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PROTHALLIA OF THE CYATHEACEAE

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(WITH ONE HUNDRED AND EIGHTY-SIX FIGURES)

Introduction

The family Cyatheaceae is listed by CHRISTENSEN (7) as consisting of seven genera and 456 species. Of these, three genera and 426 species belong to the Cyatheae, one monotypic genus to the Thyrsopterideae, and three genera and twenty-nine species to the Dicksonieae. Scarcely twenty species have been described in the gametophytic stage, and about half of these have been studied only in the germination stage or have been the subject of experiments in germination. BAUKE (1) gave a complete history of the gametophyte, based on a study of *Cyathea medullaris*, *Alsophila australis*, and *Hemitelia spectabilis*, with a less complete study of *Balantium antarcticum*, *Cibotium schiedii*, a second *Alsophila*, and a second *Cyathea* species. WIGAND (30) in 1854 gave an account of the antheridium and noted the multicellular hairs on the prothallium. KNY (15) reported on the germination, development of prothallium, and structure of the antheridium of *Cibotium schiedii*. HEALD (12), SCHULTZ (24), LAAGE (16), and LIFE (18) gave accounts of the relation of light to germination of spores, based in part on species of the Cyatheaceae. HEIM (13) reported on some experiments on the influence of light on the formation of sex organs, using among other species two of the Cyatheaceae, *A. australis* and *Balantium antarcticum*. HEIM also discussed the characters of the gametophytic stage

and their value in classification. GOEBEL (11) discussed the prothallia of *B. antarcticum*, *Hemitelia gigantea*, and *H. walkerae*, particularly with reference to the production of multicellular hairs and the branching of the prothallia. SCHLUMBERGER (23) gave an account of a study of the antheridium of three species of the Cyatheaceae (*Cyathea dealbata*, *Hemitelia aspera*, and *Cibotium schiedii*), and made a comparison of the antheridium and multicellular hairs of the Cyatheaceae with those of certain species of the Polypodiaceae. STEPHENSON (25) reported on young stages and the antheridia of *Dicksonia squarrosa*, *Cyathea dealbata*, *C. medullaris*, and *C. cunninghamii*.

The present paper is the result of an attempt to make a comprehensive study of representative species of the Cyatheaceae, to ascertain the range of type within the family and to obtain light on the origin of the family. The similarity of the gametophyte to that of the Polypodiaceae has probably received sufficient emphasis, but the similarity to more primitive groups has received relatively little. The work was begun before the appearance of the paper by BOWER (4) in 1913, presenting the theory that the family is polyphyletic and consists of representatives of two distinct lines, the Superficiales and the Marginales, which have reached approximately the same stage in evolution. In the light of this theory it has been particularly interesting to note to what extent the evidence given by the gametophyte suggests a polyphyletic origin.

This work has been carried on over a period of eighteen years, and the results are based on more than sixty cultures, each from a different collection of spores. Each culture was carried for at least four months, most of them from eight months to a year, several for two years, and two for five years. All species were brought to the stage of the production of archegonia and sporelings. For the sake of comparison, cultures were made of representatives of the Osmundaceae, Gleicheniaceae, Schizaeaceae, and Polypodiaceae.

The spores used for culture were obtained from many sources. The writer wishes to express her indebtedness to the following: Dr. N. L. BRITTON and Dr. M. A. HOWE of the New York Botanical Garden; Mr. L. A. BOODLE of the Royal Botanic Gardens, Kew; the Director of the Botanic Gardens, Edinburgh; the Director of

Horticultural Hall, Fairmount Park, Philadelphia; Dr. F. E. STOKEY for collections in Madeira; Mr. OTTO DEGENER for collections in the Hawaiian Islands; Mr. D. WATT for collections in Jamaica; and also to Mr. C. A. WETHERBY of the Gray Herbarium, Cambridge, for the determination of *Cibotium barometz;* and to Dr. W. R. MAXON of the National Herbarium, Washington, for the determination of all other species used in this investigation.

Method

In raising fern prothallia it is necessary to take precautions to obtain pure cultures. Naturally it is necessary to avoid contamination by fungi and algae, both of which are apt to flourish under conditions which are favorable to fern prothallia. It is equally important to avoid the introduction of the spores of other species of ferns. In greenhouse material especially, one is apt to find spores of other ferns deposited on the leaves, and unless these are removed it may happen that the prothallia of the desired species will be crowded out by the more rapidly growing prothallia of undesired species; but of course the chief objection is the inability to separate a mixed culture until it is too late. In the case of greenhouse material a method referred to in an earlier paper (**27**) was found satisfactory. Portions of fertile leaves were collected when the sporangia were ripe but had not yet opened; each portion was carefully brushed with a camel's hair-brush while under running water. Few, if any, foreign spores will remain if this process is carried out thoroughly. Even after these precautions are taken it is desirable to have some checks on the purity of the cultures. The spore coat remains attached to the young prothallium for a considerable time, and this is an aid in judging the purity of young cultures. The uniformity shown by a culture in its rate of growth and development is a good indication of its purity. The real test, of course, is the production of mature sporophytes true to type, but the production of a considerable number of uniform sporelings is also a good criterion. While not all the cultures discussed in this paper were carried to the mature stage of the sporophyte, they were in enough cases to warrant the assurance that the results are based on pure cultures. All the cultures of the first two years were subsequently duplicated.

It seems probable that some of the lack of harmony in the results of work on fern prothallia may be due to the use of cultures derived from greenhouse material, and that in these cases there was not sufficient care taken to avoid the introduction of foreign spores. It is possible that some may be due to error in identification of the material from which the culture was derived. Some of the difficulty may be due to lack of uniformity in methods of culture and the absence of a definite test as to what constitutes favorable or normal conditions for growth.

Germination stages were obtained on distilled water, on tap water, on porous crock standing in water, on peat, and on soil (a mixture of leaf mold and sand). The same types were produced in all cultures but the cells were usually longer in water cultures; the water culture is satisfactory for the earliest stages only. The peat cultures were sometimes slower than those of the soil, but the same type of prothallium was produced on both. A comparison of the results led after a time to the use of peat exclusively for the later stages. Peat cultures require less frequent attention than soil cultures, as peat retains water longer and stays more uniformly moist. The prothallia are cleaner and accordingly easier to handle. The peat used was for the most part black Nantucket peat which is rather hard and compact, but some of the later cultures were made on Wisconsin peat which is lighter and looser. The peat is improved by being boiled eight or ten times with changes of water. No experiments were made to determine the most favorable degree of acidity, but many prothallia were found to grow satisfactorily in peat which had a pH of 4.6.

The prothallia were raised in low stender dishes or crystallizing dishes 6–12 cm. in diameter and 3–5 cm. high, with a layer of peat 1–2 cm. deep. Such a culture does not require watering more than once in three or four weeks. The ordinary flat stender cover is not satisfactory as it is too easily dislodged; the overlapping type is better, and it was found that one plate of a petri dish of an appropriate size made a very convenient cover. Boiled tapwater was used for watering, as it was found that boiling the water reduced the danger of infection from algae. Cultures which were infected by fungi were treated with potassium permanganate. At first this

was carefully measured according to the proportions given by TWISS (**28**), 0.15 gm. in 3.5 liters of water, as recommended by LANG, but equally good results were obtained by using the "solution decidedly pink" to which she refers. In cases of bad fungal infection the prothallia were washed in the solution, transferred to freshly sterilized dishes of peat, and given a daily application of the solution until either the fungus or the prothallia died. It is easier to get rid of fungi than of algae. Rinsing the prothallia in boiled water and transferring them to fresh peat often enables the prothallia to keep ahead of the algae but it does not effect a cure.

The cultures were kept in a laboratory in which the temperature ranged from 17° to 24° C. They were kept near a north window where they received the maximum amount of diffused light available. Direct sunlight was found to be unfavorable. Prothallia which were kept in a shaded window or at a distance of 2 or 3 m. from the window showed many irregularities, and did not follow what may be called a normal course of development. In this paper conditions which resulted in a rapid development of the typical heart-shaped prothallium, with an early formation of the cushion and of archegonia are referred to as favorable. Conditions which caused the development of ameristic prothallia, elongated pale prothallia, prothallia which never progressed beyond the antheridial stage, or prothallia which reverted to the antheridial stage after having initiated the production of archegonia are referred to as unfavorable, since such conditions would not lead to the production of sporophytes.

Paraffin sections were made of all species studied. Several killing and fixing agents were tried, but formalin alcohol (6 cc. commercial formalin in 94 cc. 50 per cent alcohol) was on the whole the most satisfactory, although good results were obtained in some cases with a weak solution of chromo-acetic acid (1 gm. chromic acid, 3 cc. acetic acid, 300 cc. water). The stains used were Flemming's triple stain, Haidenhain's iron-alum haematoxylin counterstained with orange G, and a combination of safranin and anilin blue.

For the study of antheridia fresh material was found the most satisfactory, although in some cases it was helpful to use material which had been bleached in a weak solution of potassium hydroxide.

Cyatheae

The following list gives the fifteen species of the Cyatheae investigated, together with the length of time each was kept in culture.

Lophosoria quadripinnata (Gmel.) Bower (*Alsophila quadripinnata* [Gmel.] C. Chr.[1]) 10 months, 5 years
Alsophila armata (Sw.) Pr. 10 months
Alsophila aspera (L.) R. Br. 1 year, 15 months
Alsophila atrovirens (Langds. & Fisch.) Pr. 14, 15 months
Alsophila cooperi F. Muell. 9, 10, 16 months
Alsophila excelsa R. Br. 4, 10 months
Hemitelia horrida (L.) R. Br. 5, 8 months
Hemitelia parvula (Jenm.) Bak. 11 months
Hemitelia smithii (Hk. fil.) Hk. 5 months
Cyathea arborea (L.) Sm. 7 months, 1 year
Cyathea dealbata (Forst.) Sw. 1 year
Cyathea medullaris (Forst.) Sw. 10 months
Cyathea muricata Willd. 4, 6 months
Cyathea nigrescens (Hk.) J. Sm. 10 months
Cyathea tussacii Desv. 6 months

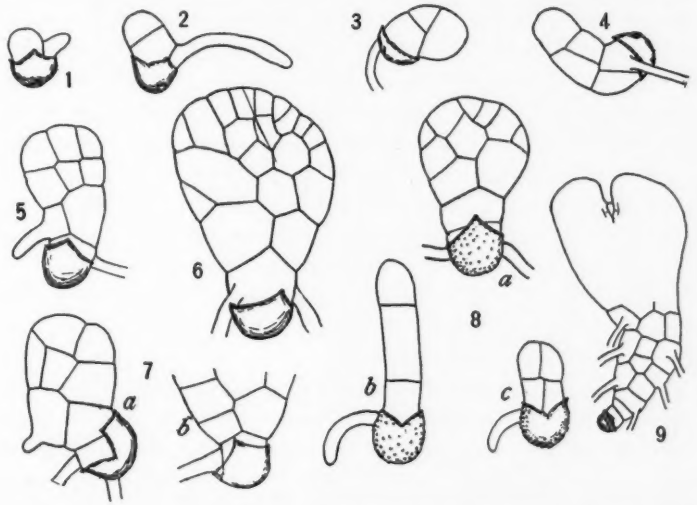
Vegetative structure

The spores of all the species studied are of the tetrahedral type but vary in size, color, and texture of wall. Some are smooth (*Alsophila excelsa*); those of *A. cooperi* are covered with spines; those of *Hemitelia horrida* have peculiar disks in the wall; while those of *Lophosoria quadripinnata* have a characteristic ridge. In general, germination was better when the spores were fresh, but in some cases a delay of 3–4 months made little or no difference; in a few cases germination was better after a resting period of several months. As a rule the percentage of viable spores decreased after 3–4 months and germination became slower. No experiments were carried on to determine how long the spores retained their viability, but in several cases it was possible to germinate spores 12–15 months old. In the case of most spores a change in color of the contents indicated that germination was about to take place, but in some

[1] In the nomenclature of this species Bower has been followed instead of Christensen.

cases the first indication was the rupture of the spore coat at the apex of the tripartite ridge.

The first wall is parallel to the axis of the spore and separates the first prothallial cell from the first rhizoid (fig. 1). At first the rhizoid elongates more rapidly than the prothallial cell. With the growth of the prothallial cell a wall is formed which is more or less oblique to the wall cutting off the rhizoid cell (fig. 2). The prothallial cell



FIGS. 1–9.—Figs. 1–4, *Hemitelia horrida;* figs. 5, 6, *Alsophila excelsa;* fig. 7, *Cyathea muricata;* fig. 8, *A. cooperi,* ×165; fig. 9, *Lophosoria quadripinnata,* ×60.

broadens as well as lengthens, pushing to one side the primary rhizoid cell (figs. 2, 7*b*, 8*b*). The next division may be a longitudinal division of the terminal cell, an oblique, or a horizontal division. Under favorable conditions the thallus broadens early; a filament of four or five vigorous cells, such as is commonly found in the Polypodiaceae, is unusual. If such a filament does form, all the cells except one (perhaps two but rarely three) at the base will divide longitudinally or obliquely at about the same time. If the culture is crowded or in weak light more or less depauperate filaments with long cells are formed (fig. 8*b*). Typically the only cell of

the initial filament which persists without longitudinal or oblique division is that which remains in part within the spore coat (figs. 5, 7*b*). Fig. 8*a* shows a prothallium of *Alsophila cooperi* with a 2-celled filament at the base, and fig. 9 one of *L. quadripinnata* with three, but it will be noted that the aspect of the prothallia is not that of the early stages of polypods. The young prothallia are distinctly shorter and broader than those of the Polypodiaceae grown under what may be called favorable conditions. The same results were obtained in many series of cultures. No notable differences were found between germination in June and December, certainly no difference which could be correlated with length of day or intensity of light. It requires a reduction below the intensity of maximum diffuse light of December in latitude 42° to produce filaments of the depauperate type. It should be noted, perhaps, that the presence of snow on the ground in Massachusetts during the winter months makes the reduction in light less than might be expected. Germination was of the same type whether on soil or peat, and for the most part whether on water or on a solid substratum. Even in the early stages, however, the water cultures were distinctly less vigorous than those on soil or peat.

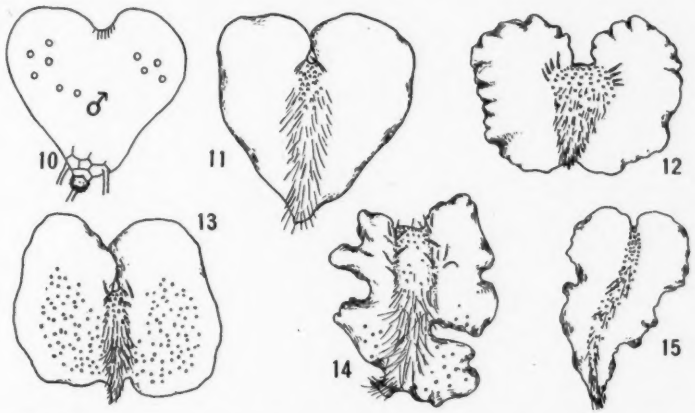
The results given here are similar to those obtained by BAUKE, who stated that the prothallia of the Cyatheaceae differ from those of the Polypodiaceae in the earlier formation of the plate and in the fact that no filament is left at the base of the prothallium.

The apical cell appears early, sometimes in the 4- or 5-celled stage but ordinarily later (fig. 8*a*); subsequent divisions are shown in fig. 6. The apical cell is later replaced by a group of initials. The production of antheridia usually begins about this time (fig. 10). In general the appearance of antheridia is distinctly later than in the polypods; or perhaps it is better to say that no indication was seen of the ability to produce antheridia at the very early stages (5- to 25-celled) such as is frequently found in the polypods. The cushion develops in the usual manner.

The mature prothallium of the Cyatheae is the typical heart-shaped structure such as prevails among the leptosporangiate ferns. To attain this form the prothallia must have ample space, a suitable amount of moisture, and a favorable intensity of light. While the

prothallia of the various species studied conform to the same general type, they are by no means uniform, but vary in color, size, proportion, thickness and width of rib, and amount of cutinization both on the surface and on the sex organs.

The prothallia of *Cyathea* were found to approach most closely to those of the polypods, being broader and shorter, with a smaller and thinner cushion than those of *Alsophila* and *Hemitelia*. The prothallia of *Lophosoria* were relatively thin and delicate, but are

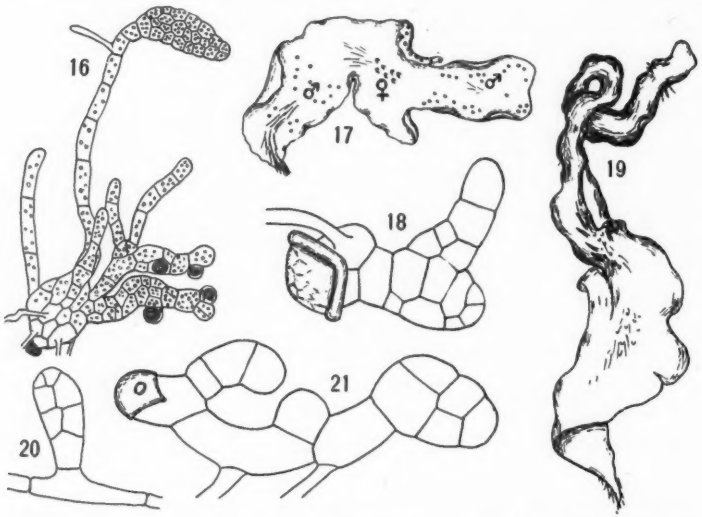


FIGS. 10–15.—Fig. 10, *Alsophila armata*, ×40; fig. 11, *Cyathea tussacii*, ×6; fig. 12, *Hemitelia smithii*, ×5; fig. 13, *A. cooperi*, ×5; fig. 14, *A. excelsa*, ×5; fig. 15, *Lophosoria quadripinnata*, ×5.

too long to suggest the polypod type. The prothallia of *Lophosoria* were studied in two sets of cultures, one of which ran for two years and the other for five, but at no time did the prothallia become heavy or robust in appearance. They produced healthy gametes and numerous sporelings, but they never approached the massive types found in some species of *Alsophila* and particularly in *Cibotium barometz*. Even those which developed branched cushions (fig. 24) were rather delicate. It is quite possible that the culture conditions were not so favorable for this species as for the others, although they brought the prothallia to maturity.

Forking of the apical meristem with subsequent branching of the

cushion was found to occur *L. quadripinnata*, *A. armata*, *A. atrovirens*, *H. horrida*, *H. parvula*, and *C. dealbata* (figs. 22–25). This results in two or more regions of archegonial production. The tendency to branch is shown earlier and more frequently in *Hemitelia* than in the other genera. GOEBEL (11) has discussed its occurrence in *H. gigantea* and *H. walkerae*. In some plants of *H. parvula* (fig.

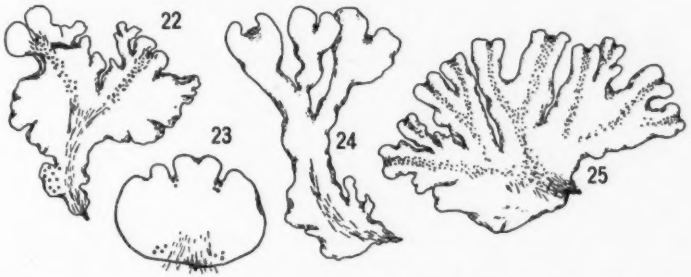


FIGS. 16–21.—Fig. 16, *Alsophila excelsa*, ×40; fig. 17, *Cyathea arborea*, ×8; fig. 18, *Lophosoria quadripinnata*, ×165; fig. 19, *Cyathea* sp., ×8; fig. 20, *A. cooperi*, ×80; fig. 21, *Hemitelia horrida*, ×200.

25) the branching was almost as regular as in *Riccia*, and resulted in a broad fan-shaped prothallium very suggestive of a liverwort. In *Hemitelia* forking may occur at so early a stage (fig. 23) that it seems probable that it may occur frequently in nature, but in the other forms the growth of the prothallium would ordinarily be checked by embryo formation before forking would occur.

The thickness of the cushion varies apparently both with the species and with the cultural conditions. In old prothallia which were vigorous and healthy there was a tendency for the rib to develop more strongly than the wings. In such a prothallium as that shown

in fig. 14 the wing development has not kept pace with the rib and in some prothallia the rib extends as a heavy flattened or rounded projection beyond the wings. Fig. 19 shows a 14-months old prothallium of a species of *Cyathea* from Jamaica (probably *C. serra* but the material was insufficient for positive determination) which gives such a case of rib development. The growth did not give the slightest indication of being apogamous. Such an elongated rib may become more or less erect, or in the case of more robust prothallia with a broader heavier rib, the extension may curl under or backward to such an extent that the morphologically dorsal surface be-

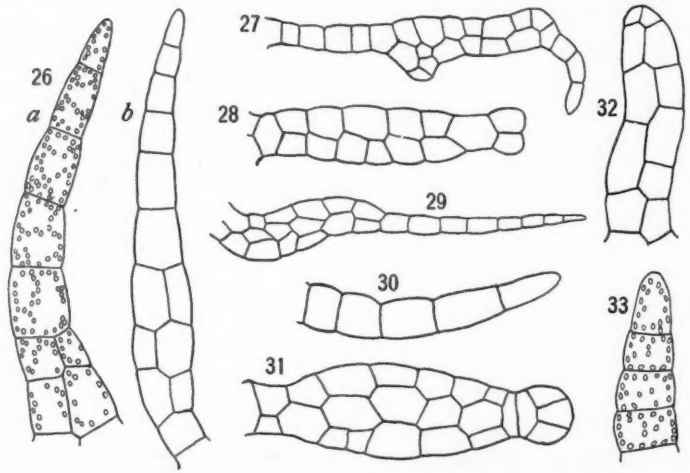


FIGS. 22–25.—Fig. 22, *Alsophila armata*, ×5; fig. 23, *Hemitelia horrida*, ×5; fig. 24, *Lophosoria quadripinnata*, ×6; fig. 25, *H. smithii*, ×2.5.

comes the physically lower, and completely takes over the formation of rhizoids and archegonia. In some cases when fertilization did not occur and the prothallia were allowed to grow indefinitely the wings tended to become irregular and were more or less curled and crisped (figs. 12, 14), suggesting somewhat the *Gleichenias* described and figured by CAMPBELL (**6**), but no species of the Cyatheae included in this investigation showed such fine examples of it as did some of the Dicksonieae.

In weak light the tendency to elongate was very much pronounced, and with this there might be a noticeable change in the thickness of the thallus and then in the type of sex organ produced. Fig. 17 shows a prothallium of *Cyathea arborea* which produced antheridia, then for a period archegonia only, then antheridia only. This was found to be definitely related to the intensity of light and

consequently to nutrition, as was shown by WUIST (29) in the case of *Onoclea struthiopteris*. In certain species (*A. excelsa*) there is a strong tendency in weak light for prothallia to develop as slender branching ameristic structures which produce antheridia only, but in other species this is much less common. The branched prothallia shown in figs. 18 and 21 were from young crowded cultures. Fig. 16 shows a plate which has given rise to numerous filaments, one of which has developed a plate at its tip. In fig. 20 is shown a plate which has arisen laterally on a long filamentous prothallium.

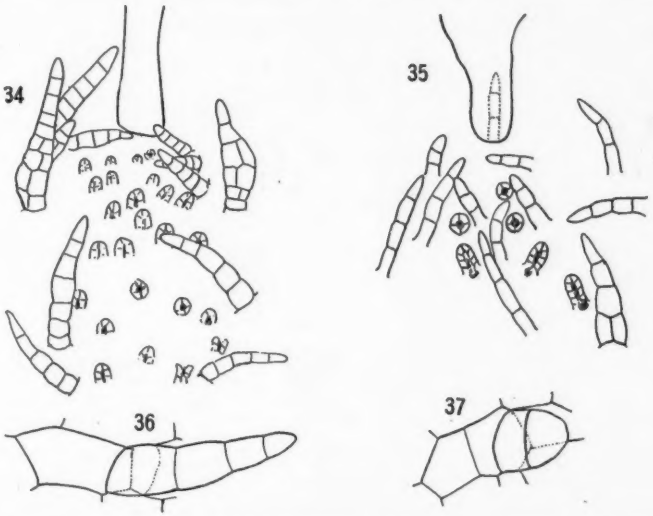


FIGS. 26–33.—Figs. 26, 28, *Alsophila excelsa*, ×180; fig. 27, *Hemitelia horrida*, ×160; figs. 29, 31, *A. aspera;* fig. 29, ×45; fig. 31, ×120; fig. 30, *Cyathea dealbata*, ×120; fig. 32, *C. nigrescens*, ×90; fig. 33, *A. atrovirens*, ×165.

HAIRS.—References to the multicellular hairs of the Cyatheaceae are found in most of the literature dealing with mature prothallia. The hairs were first mentioned by WIGAND (30), and were later described by BAUKE (1) as "borstenförmigen Haaren." In some accounts they are said to be characteristic of the family; in others, of a particular genus, for example, *Alsophila*. According to the present investigation they are limited to the members of the subfamily Cyatheae as given by CHRISTENSEN.

The multicellular hairs show a wide range both in size and shape.

Those on young prothallia are uniseriate and may not be more than three or four cells long (fig. 33), while on old but vigorous prothallia they may be twenty to thirty cells long with an actual length of 2 mm. or more (figs. 46–48). Figs. 26, 29, 30 illustrate the most common types on prothallia 2–5 months old. The base is ordinarily more than one cell in width, but the tip is usually uniseriate and pointed; the broadened tip (figs. 28, 31, 32, 47) is relatively infre-

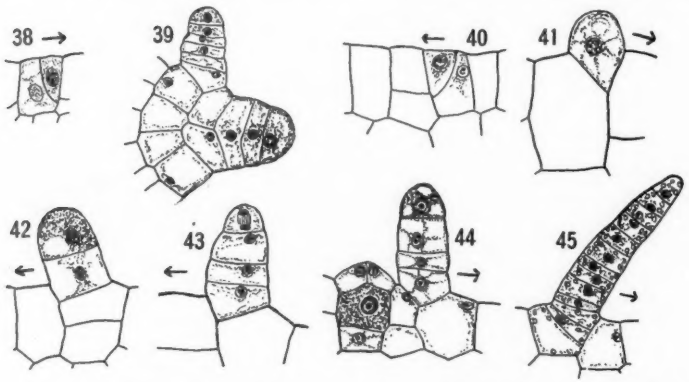


FIGS. 34–37.—Fig. 34, *Alsophila atrovirens*, ×48; fig. 35, *A. aspera*, ×48; fig. 36, *A. aspera*, ×165; fig. 37, *A. atrovirens*, ×230.

quent. Figs. 46–48 are from hairs on large prothallia. Such hairs are easily seen with the unaided eye and may give a shaggy appearance to the region of the cushion. The width of the hair usually ranges from one to three cells, but may reach eight to ten; they are ordinarily one cell thick, but large hairs may be several cells thick at the base (figs. 46, 47). All the cells of the hair may be capable of division for a considerable period. It will be seen from fig. 49 that the cells in the region above the base may persist in the meristematic condition after the cells at the base and the tip have begun to mature. In no case was a glandular tip observed nor was there any

evidence of glandular character. The cells of the hair are abundantly provided with chloroplasts and are as green as the prothallium which bears them. The cells of the prothallium at the base of the hair persist as healthy green cells.

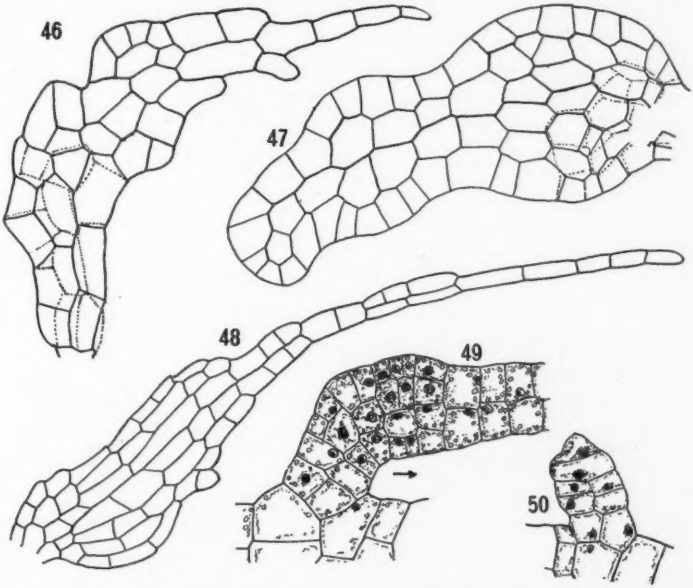
The hairs are found on both the dorsal and ventral surface of the prothallium but not on the margin or near it. On the ventral surface they usually develop along the sides of the cushion and on the adjacent regions of the wings (fig. 34), but they may be found among the archegonia (fig. 35). On the dorsal surface they are usually



FIGS. 38–45.—Figs. 38, 39, *A. armata;* fig. 38, ×285; fig. 39, ×260; fig. 40, *Cyathea arborea,* ×300; fig. 41, *A. cooperi,* ×285; fig. 42, *H. horrida,* ×285; figs. 43, 44, *A. excelsa,* ×285; fig. 45, *C. medullaris,* ×200.

found on the cushion, spreading occasionally to the wings. The formation of hairs seems to be related to the stage of maturity and vigor of the prothallium. They do not appear ordinarily until after the formation of the cushion. In species of *Alsophila* they may appear before any archegonia have formed, but typically they do not appear until one or more archegonia have matured. In rare cases they may appear on prothallia which have not begun to thicken and which bear only antheridia. Hairs were later in appearing in *Cyathea* and *Hemitelia* than in *Alsophila.* Hairs were found on a few prothallia only in the case of *Lophosoria;* they were late in appearing, and were short and uniseriate. As noted, the prothallia of *Lophosoria,* while as long-lived as any other species in culture,

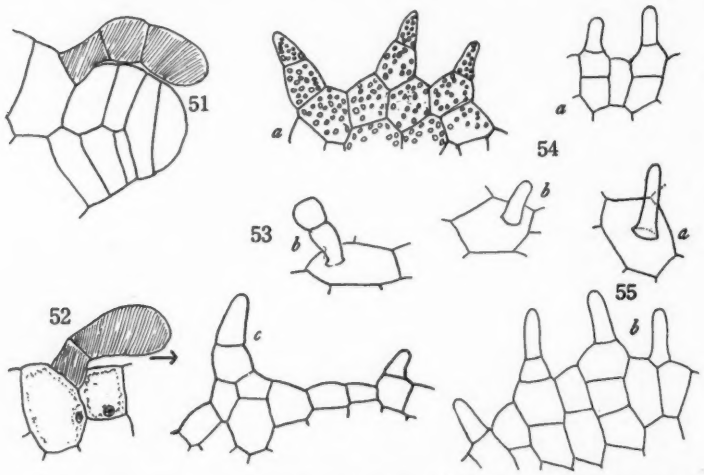
never appeared to be vigorous. It may be that the meager and belated production of hairs is to be explained in this way, but it does not seem probable. As GOEBEL (11) has pointed out, it is very likely to happen in certain species (*L. quadripinnata*, species of *Cyathea* and *Hemitelia*) that prothallia may mature, bear sporophytes, and disintegrate without having produced any hairs.



FIGS. 46–50.—Figs. 46, 47, 50, *Alsophila atrovirens*, figs. 46, 47, ×80; fig. 50, ×210; figs. 48, 49, *Cyathea medullaris;* fig. 48, ×50; fig. 49, ×250.

The origin of the multicellular hair is shown in figs. 38, 40, 41, in which the arrow indicates the position of the apical region of the prothallium. It will be seen that it arises from a wedge-shaped cell cut off from the anterior side of a prothallial cell near the apical region, being formed by a wall which is oblique to the surface of the prothallium. The later growth of the mother cell and of the adjacent prothallial cells, as well as that of the hair, gives the hair a wedge-shaped base (figs. 39, 41–45, 49). Fig. 50 shows the base of a hair

in a section cut across the cushion. Surface views of hairs arising from the thallus are shown in figs. 36, 37. This type of origin of a trichome was reported by LEAVITT (**17**) for the root hairs of certain species of angiosperms, and by STOKEY (**26**) for those of certain species of *Lycopodium*. It is unlike the origin of rhizoids which form from a projecting papilla near the center of an ordinary prothallial cell. It is also distinctly different from the origin of the hairs which



FIGS. 51–55.—Figs. 51, 52, *Gleichenia pectinata*, ×285; fig. 53, *Phyllitis scolopendrium*, ×145; fig. 54, *Dryopteris filix mas*, ×145; fig. 55, *Woodsia obtusa*, fig. 55*a*, ×165; fig. 55*b*, ×180.

are found on many species of the Polypodiaceae (for example, *Dryopteris filix mas*, fig. 54). Fig. 39 shows the relation of the hair to the apical cell in *A. armata*, and indicates that the wedge-shaped base is not due to a secondary division of the trichome-forming cell, as is sometimes the case in the Polypodiaceae (*Woodsia obtusa*, fig. 55*b*).

BECK (**2**) described and figured multicellular hairs on the prothallia of *Phyllitis scolopendrium* (*Scolopendrium vulgare*), comparing them with those of the Cyatheaceae. SCHLUMBERGER (**23**) con-

firmed this work. BECK's drawings of multicellular hairs indicate that the origin of the hair in *Phyllitis* corresponds to the usual polypod type. In the writer's cultures of *Phyllitis* the hairs present were, for the most part, of a type which indicates only a small departure from the usual polypod type (fig. 53). Very few consisted of more than two or three cells. The usual type was simple one-celled or glandular. In a species so notable for the variations of its sporophyte, however, it might well be expected that there would be considerable range in the gametophyte, and that some strains would produce better crops of hairs than others. What is more probable is that the difference is due to the fact that old prothallia were not studied. The hairs in *Phyllitis*, unlike those in the Cyatheae, are found on the margin as well as widely distributed over both surfaces.

SCHLUMBERGER (**23**) studied the hairs of *Woodsia* and *Diacalpe*, tracing a relation between them and the type of the Cyatheaceae, and suggested that they were a transitional form. His drawings show that in origin they are typically polypod and do not arise from a special initial. This has been confirmed in cultures of *Woodsia ilvensis* and *W. obtusa* (fig. 55).

The origin of the multicellular hairs in *Gleichenia pectinata* (figs. 51, 52) is the same as that of the hairs of the Cyatheae. The hair in *Gleichenia* arises from a special initial. CAMPBELL (**6**) gives an illustration (fig. 107) of a hair of *G. polypodioides* which is similar to fig. 51, and indicates that the hair is formed in the same way. He also states that the hairs occur on or near the cushion among the archegonia. This is also the distribution in *G. pectinata*.

The hairs which GOEBEL (**10**) has described as present on the prothallia of *Loxsoma cunninghamia* are very suggestive of those of the Cyatheae, both in form and distribution. Their origin is not given, and Professor GOEBEL said in a recent letter that he had unfortunately no material from which this could be determined. They conform so completely to the cyatheoid type in form and distribution that it seems highly probable that they agree in origin. This would seem to be an argument of some weight in allying *Loxsoma* with the Gleicheniaceae-Cyatheae line.

REPRODUCTIVE STRUCTURES

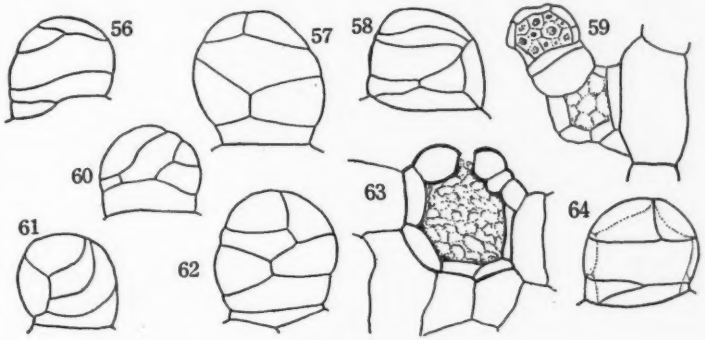
The prothallia are typically monoecious, but, as in the case of many ferns, there may be a tendency toward dioecism. In certain species, as *Hemitelia smithii*, the production of antheridia ceases when archegonia begin to appear; in others the production is more or less gradually reduced, ultimately stopping (fig. 14); in *A. cooperi* the formation of archegonia causes little or no change in antheridial production (fig. 13).

ANTHERIDIUM.—The main features of the structure of the antheridium of the Cyatheae were established in 1876 by BAUKE (1), whose work was chiefly on *Cyathea medullaris*. His account, which has since served as the basis of all general accounts of the group, was supplemented in 1907 by STEPHENSON (25), who had investigated the antheridia of *C. dealbata*, *C. cunninghamia*, *C. medullaris*, and *Dicksonia squarrosa*, and described them as being similar to the "complex normal type" described by BAUKE and showing the same variety of reduction forms. SCHLUMBERGER, in his study of transition forms between the Cyatheaceae and the Polypodiaceae, investigated *Cyathea dealbata*, *H. aspera*, and *Cibotium schiedii*.

In the present paper an attempt has been made to study the range in structure of the antheridium of this family, and to ascertain if there is any variation in type which may be correlated with the divisions of the family. In the papers mentioned the antheridia of less than ten species have been described, belonging chiefly to the Cyatheae. It should be noted that *Cyathea*, which is regarded as the most advanced genus of the Cyatheae, has received most of the attention. It has been found that there is in every species a certain amount of variation from the type given as characteristic of the family. This seems to be related to the nutrition of the thallus, ordinarily, since the antheridium varies with the age and type of the thallus, that is, whether a normal heart-shaped thallus, a lateral shoot, or a filamentous ameristic prothallium. The present work indicates that in addition to these variations within any given species, there are other tendencies which seem to have some genetic significance.

The antheridia form ordinarily on the ventral surface; they may develop on the dorsal surface but are not marginal in the case of

typical heart-shaped prothallia; on filamentous ameristic prothallia they may be lateral or terminal (fig. 16). Fig. 65 shows an antheridium initial of *A. cooperi* on the anterior side of one of the wing cells. The type of antheridium which may be regarded as characteristic of the family was described by BAUKE with great care and completeness. The wall consists of five cells: a basal cell which is usually wedge-shaped; a lower ring (funnel) cell which is attached longitudinally to the antheridial wall by a connecting or binding wall (BAUKE's "Zwischenmembrane"); an upper ring cell; a crescent-shaped and an elliptical opercular cell formed by the division of the

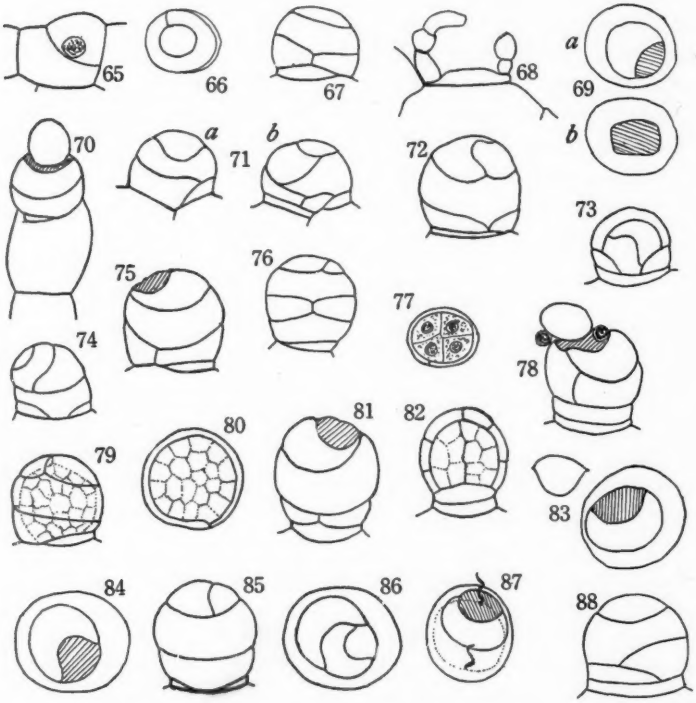


FIGS. 56–64.—*Lophosoria quadripinnata*, ×300.

primary cover cell. In general the antheridia on well developed prothallia of *Alsophila*, *Hemitelic*, and *Cyathea* correspond to this type (figs. 67, 72, 75, 81, 85); the antheridium of *Lophosoria* usually suggests a more primitive type (figs. 56, 58, 64), but the characteristic antheridium of the Cyatheae is also found (fig. 57).

In typical antheridia the stalk cell is wedge-shaped and relatively thin, extending ordinarily from half to three-fourths across the base (figs. 66, 67, 75, 79) or even farther (fig. 85), but seldom completely across. In rare cases it divides vertically. In small antheridia, especially those on filamentous prothallia, the stalk cell may be absent. Sometimes there are two stalk cells (fig. 82). In stained preparations the stalk cell is strongly marked by the modification of its walls, which are often somewhat thicker than the other walls and

are usually heavily cutinized, so that the basal cell does not absorb water and swell as readily as do the other cells of the antheridium. The pronounced cutinization usually extends to the lower part of



FIGS. 65–88.—Figs. 65–72, *Alsophila cooperi;* figs. 73, 74, *A. armata;* figs. 75–77, *A. atrovirens;* figs. 78, 79, *A. excelsa;* fig. 80, *A. aspera;* fig. 81, *C. muricata;* fig. 82, *C. arborea;* fig 83, *C. medullaris;* fig. 84, *C. dealbata;* figs. 85–87, *Hemitelia horrida;* fig. 88, *H. smithii;* all ×300.

the funnel cell and may extend farther. The presence of the stalk cell is one of the characteristics which distinguishes the antheridium of the Cyatheae from that of the Polypodiaceae, and associates it with that of more primitive families.

The structure of the lower ring cell is another character which differentiates the Cyatheae from the Polypodiaceae, since the length-

wise attachment of the ring cell is not found in the polypods. Fig. 66 shows a young antheridium viewed from above in which there is a basal cell extending somewhat more than half across the base, the lower ring cell with its binding wall, and the more or less cylindrical cell in the center from which the dome cell is later cut off. Figs. 77 and 80 show the lower ring cell in cross-section. It sometimes happens that the two ends of this cell do not come together but connect with the antheridial wall at a noticeable distance apart (fig. 73). As BAUKE pointed out, the presence of the binding wall checks growth of the outer wall of the antheridium in the region of attachment, making the cell (and antheridium) asymmetrical (figs. 67, 68, 72, 79, 81). The second ring cell is formed from the dome cell and is more symmetrical. In some cases a binding wall is seen in this cell (fig. 78). It is possible that in such cases the second ring cell is formed in the same way as the first. BAUKE, however, thought it more probable that the wall was a secondary formation. Sometimes three ring cells are formed (fig. 76), making the antheridial wall a 6-celled structure.

The primary cap cell usually divides once (figs. 68, 69*a*, 75, 76, 78, 79, 81–85), but in large antheridia it may divide twice (fig. 86). In small antheridia it may remain undivided and is then identical with the polypod cap cell (figs. 69*b*, 70). The primary cover cell is formed by a circular wall which may be near the center of the dome cell, making a relatively symmetrical antheridium (figs. 76, 79, 85); or it may form to one side, making a distinctly asymmetrical antheridium (figs. 78, 87). Occasionally, especially in *Cyathea*, the walls of the opercular cell are modified and highly refractive (fig. 82). The opercular cell is thrown off at dehiscence (figs. 70, 78, 83) and, unlike the delicate opercular cell of the Polypodiaceae, may persist intact for many hours.

There are many variations from the typical antheridium. Reduction forms are frequent on filamentous ameristic prothallia. Additional cells may be present. In the antheridia shown in figs. 76, 82, and 86 the additional cell present may be regarded merely as a duplication of the lower ring cell, the basal cell, and the opercular cell respectively; but in such antheridia as those shown in figs. 71 (*a* and *b* are two views of the same antheridium), 73, 74, and 88 the

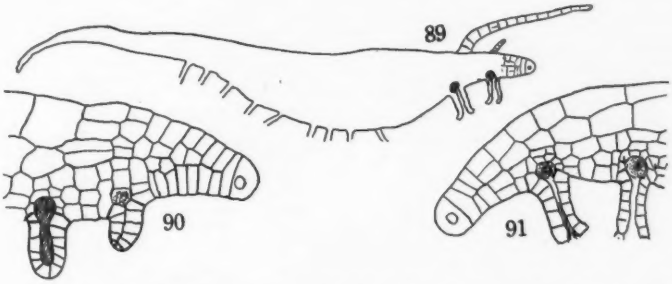
usual terminology does not fit. This type of irregularity is suggestive of the antheridia of primitive ferns.

The antheridia of *Lophosoria* stand somewhat apart from those of the other members of the Cyatheae under investigation. They are somewhat larger on the average, and are more irregular in aspect. The number of sperms in cross-section ranges from eighteen to twenty-eight, averaging twenty-three or twenty-four, while in *Alsophila* and *Hemitelia* the number ranges from ten to twenty-five but is usually between fifteen and twenty. In *Cyathea* it is apt to be somewhat smaller. The larger size of the antheridia cannot be related to large prothallia, since the prothallia were rather undersized. Probably most of the antheridia in *Lophosoria* have a 5-celled wall, but the antheridium is usually asymmetrical and has the effect of being formed by a series of wedge-shaped cells. They are suggestive of the antheridia of *Gleichenia* or *Osmunda*, rather than of the Polypodiaceae. Fig. 59 shows a case of an antheridium which has proliferated and given rise to a second small antheridium. On one prothallium an imbedded antheridium was found (fig. 63). Since imbedded antheridia have been found to occur in the Polypodiaceae, as reported by BLACK (3) and FERGUSON (9), it is surprising that this is the only case found in this family.

The antheridia of *Cyathea* seem to approach those of the polypods rather more closely than do those of *Alsophila* and *Hemitelia*. They are inclined to be somewhat smaller and rather more symmetrical. BOWER (5) has emphasized the relation between the size of sporangia and antheridia. *C. dealbata* is of interest because of the small spore output. The range in size of antheridia in any species is so great that it is difficult to be positive about slight differences. The antheridium in *C. dealbata* is not large, but it can hardly be said that it is obviously smaller than that of other species of *Cyathea;* the one shown in fig. 84 represents probably the maximum size, and that is not the case for the figures of the antheridia of the other species. The difference between the antheridia of *L. quadripinnata* and those of *C. dealbata* both in size and symmetry is striking, but it is not possible to speak so positively about the various species of *Cyathea* in which differences are slight if they exist at all.

ARCHEGONIUM.—As is the case in all typically heart-shaped pro-

thallia, the appearance of archegonia depends upon the development of the cushion. In the Cyatheae archegonia do not appear as early in the development of the prothallium as in the Polypodiaceae, that is to say, the prothallium is later in arriving at maturity. In polypod prothallia mature archegonia may be found on prothallia in which the adjacent tissue is only two cells thick and there is no vegetative part any thicker. No cases of this were seen in the Cyatheae. In a few cases mature archegonia were found on a thallus in which the adjacent tissue was only three cells thick, but usually it was at least four cells thick. The prothallia shown in figs. 89–91



FIGS. 89–91.—*Alsophila cooperi:* fig. 89, ×45; fig. 91, ×105; fig. 90, *A. excelsa*, ×105.

are relatively young. Figs. 89 and 91 show sections of prothallia made eight weeks after the spores were planted; that shown in fig. 89 had ten mature archegonia and was six cells thick in the heaviest part, the other had only six archegonia. Fig. 90 shows a prothallium which had twelve archegonia.

The archegonium initial (fig. 93) appears near the apical region, usually 3–5 cells from the apex. The first division in the initial cuts off the primary neck cell (figs. 92*a*, 94) and the next forms the basal cell (figs. 94–96). Fig. 96 shows a "row of three" in *C. medullaris* formed from a wedge-shaped initial; this is a rare type in the Cyatheae. BAUKE (1) states that in the Polypodiaceae two basal cells are formed occasionally, but that in certain species of the Cyatheaceae it is the rule. That cannot be said to be the case in any species included in this investigation. While the formation of two basal

cells is of frequent occurrence (fig. 98), and even three may be formed (fig. 104), the archegonium with one basal cell is the usual type and that with two the exception. In the species of *Hemitelia* under investigation cases of two basal cells were more frequent than in the other genera; but BAUKE states that *H. spectabilis* did not produce two basal cells, while *C. medullaris* and *A. australis* did regularly. In the archegonium of *H. horrida* (fig. 102) the two basal cells were cut off obliquely. In *Lophosoria* cases of two basal cells are rare. It may be that the tendency to form two basal cells is related to the tendency of the Cyatheae to develop a thicker thallus than that of the polypods; the individual cell, as well as the prothal-

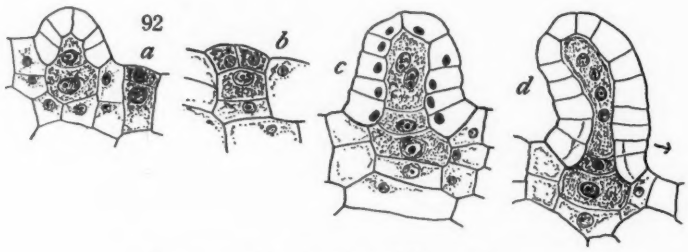
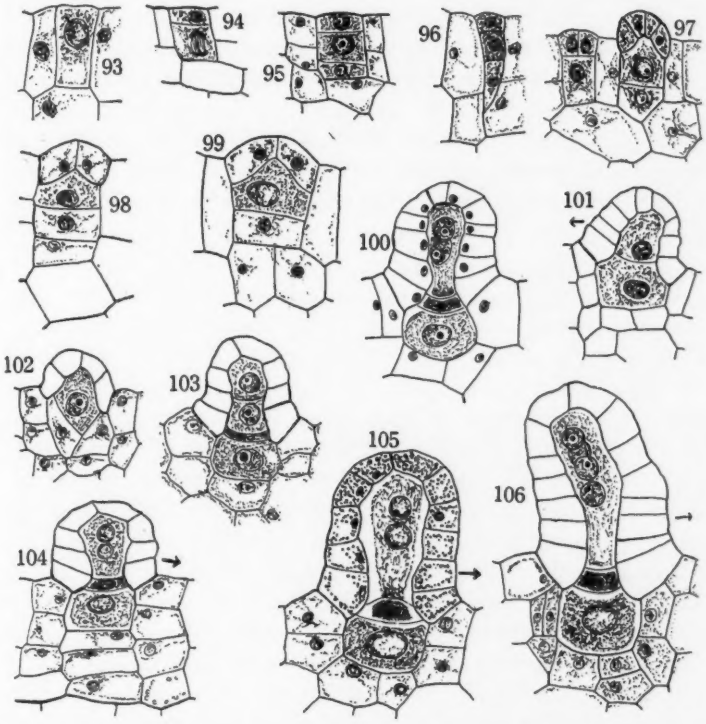


FIG. 92.—*a–d*, *Lophosoria quadripinnata*, ×300.

lium as a whole, not maturing so rapidly. The origin of the second basal cell was not determined as no mitotic figures were seen. The production of two oblique cells, which was seen several times, indicates that both may be formed from the central cell. In several cases, however, the basal cell divided vertically at an early stage (figs. 92*a*, 101), and this suggests that the second basal cell is formed by the division of the primary basal cell. ROGERS (**22**), in discussing the question of basal cells in *Lygodium*, suggests that in many cases the so-called second basal cell is not a true basal cell in the sense that it is a product of the archegonium initial. It is certainly true that many cases were seen in which a cell was present which might have been derived from the thallus beneath the archegonium initial and yet looked like a second basal cell, but in other cases the second basal cell was undoubtedly a product of the archegonium initial.

The arching of the neck usually begins with the division of the

primary neck cell into the four cells which form the initials for the four rows of neck cells; the central cell then pushes up between the neck cells (figs. 92*b*, 98, 99). Figs. 92*a*, 97, and 101 illustrate the



FIGS. 93–106.—Figs. 93, 94, 97, 98, 102, 104, 105, *Hemitelia horrida*, ×300; figs. 95, 101, *Alsophila excelsa;* figs. 96, 99, *Cyathea medullaris;* fig. 100, *A. cooperi;* fig. 103, *H. smithii;* fig. 106, *C. dealbata;* ×300.

next stages in the development of the archegonium, the further divisions of the neck cells and the division of the central cell to form the primary neck and the primary ventral cell. Figs. 92*c*, 100, 104, and 105 show the mature archegonium in which has occurred the division of the primary ventral cell to form the egg and ventral canal cell, and the division of the primary neck canal nucleus. The

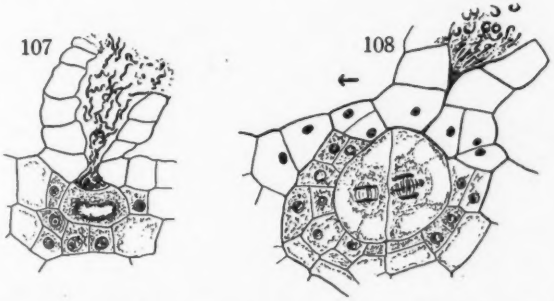
neck canal nucleus ordinarily divides once, giving the mature archegonium two neck canal nuclei, but the formation of four neck canal nuclei is of rather frequent occurrence (figs. 92*d*, 106), and cases were found in all the species studied. TWISS (28) has reported it for *Lygodium circinatum*, ROGERS (22) for *L. palmatum*, and PFEIFFER (21) for *Pteris longifolia*. Only one archegonium was found in which there was a wall separating the neck canal nuclei; this was in *H. smithii* (fig. 103).

In the character of the mature archegonium (the length and straightness of the neck) the Cyatheae ally themselves with the more primitive families, the Osmundaceae and the Gleicheniaceae, rather than with the Polypodiaceae. The necks are noticeably longer and straighter than those of polypods, with the anterior side having only one more cell than the posterior and with very little elongation of the anterior cells. The curve, when present, is usually away from the notch but may be toward the side, or even toward the notch. The position of the apical region is indicated in the illustrations by the arrow. The longer side of the archegonium is usually six or seven cells long, occasionally eight, rarely five; while in the polypods it is usually four or five, less frequently six. Archegonia which have four neck canal nuclei are usually somewhat longer than the typical archegonium, and are more likely to be curved.

In mature and even in young archegonia there is a striking difference between the contents and walls of the neck cells and those of the venter or of the other cells of the thallus. This differentiation may appear just after the first division of the primary neck cell, or it may not be evident until several divisions have taken place. In fresh material the neck cells are pale and have coarsely granular contents; in stained preparations the contents may show as conspicuously stained granules, or the contents may stain rather uniformly, suggesting a mucilaginous character. The reactions of the neck cells suggest a heavy cutinization. The reaction to killing and fixing agents is unlike that of the venter of the archegonium or the vegetative part of the thallus; it is more difficult to get effective penetration in the neck cells. This is probably the reason why formalin alcohol usually gave better results as a killing agent than chromo-acetic acid. The peculiar character of the walls is most marked in slightly faded preparations which have been stained with

Flemming's triple stain, as the neck cells retain the stain much better than the others. The archegonium in such cases may have the appearance of a cap set in the thallus. This modification of the neck is not characteristic of the Polypodiaceae, but is found in such primitive families as the Osmundaceae.

As the archegonium matures there may occur divisions in the cells surrounding the egg (figs. 106, 107); secondary divisions may also occur in the neck cells (fig. 92*d*). If water is present at this time the archegonium opens and pours out the more or less mucilaginous contents of the canal. The reaction of the sperms in the region of the archegonium is very pronounced; instead of swimming



FIGS. 107, 108.—Fig. 107, *A. cooperi*, ×300; fig. 108, *Cyathea dealbata*, ×300.

irregularly they dart toward the mouth of the archegonium and enter, often in great numbers (fig. 107), straightening and elongating as they penetrate the mucilaginous substance. For some time after fertilization the remains of sperms may be seen in the neck (fig. 108).

The first division of the fertilized egg is parallel to or in the general direction of the axis of the archegonium.

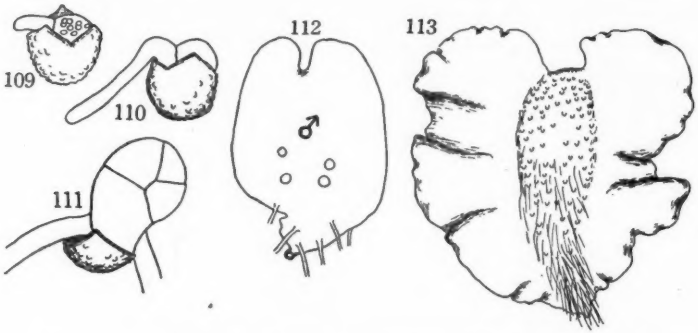
In an earlier paper (**27**) an account was given of the occurrence of apogamy in the Cyatheaceae. Since then apogamous growths have been found in *Lophosoria quadripinnata*, *A. excelsa*, *A. armata*, *H. parvula*, *C. medullaris*, and *C. dealbata*.

Thyrsopterideae

The spores of the rare endemic fern of Juan Fernandez, *Thyrsopteris elegans*, were obtained from the Royal Botanic Gardens, Kew, through the kindness of Mr. L. M. BOODLE.

VEGETATIVE STRUCTURE

The spores of *T. elegans* are of the tetrahedral type, dark brown in color, with a thick coat which has abundant irregular warty or spiny processes. They range in diameter from 210 to 275 μ, averaging 230–250 μ. Germination, with spores 2–3 weeks old, occurred in 4–7 days. The difference in rate seems to be related chiefly to temperature. Germination was obtained in spores nine months old, but



FIGS. 109–113.—*Thyrsopteris elegans:* figs. 109–111, ×165; fig. 112, ×30; fig. 113, ×7.

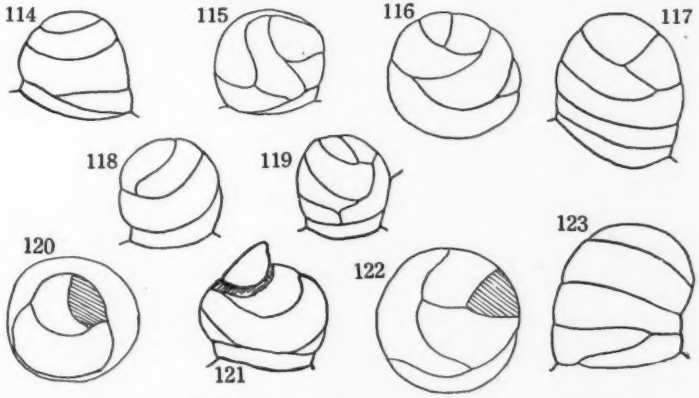
the percentage of viable spores was much lower than in fresh spores. The early stages are shown in figs. 109–111. Germination is similar to that found in the Cyatheae; there is usually no filament formed, even when spores are much crowded, but a plate is formed immediately by longitudinal or oblique divisions in the second or third cell. The prothallium elongates after the plate has formed but does not broaden rapidly at this stage (fig. 112). The prothallium when it reaches maturity (archegonial production) is like that of the Cyatheae in being relatively long and having relatively less wing than is usually found in the prothallia of the Polypodiaceae. Mature prothallia may attain considerable size, some reaching a length of 2 cm. The rib is rather wide but not especially thick, ordinarily not exceeding seven cells in thickness. The development of the rib is such that the wings are often lifted up so that the prothallium is U-shaped in cross-section. The wings may make a sufficiently

luxuriant growth to give an old prothallium a more or less ruffled appearance. Forking occurred not infrequently in prothallia over a year old, the branches being rather narrow but thick. One forking prothallium 2 cm. long had two long branches 3–5 mm. in width.

No hairs, either multicellular or of the polypod type, were found on the prothallia of *Thyrsopteris*, even on old vigorous prothallia which had 100–200 archegonia.

Reproductive structures

Antheridium.—The antheridia of *Thyrsopteris* are distinguished by their size and asymmetrical aspect (figs. 114–123). They can

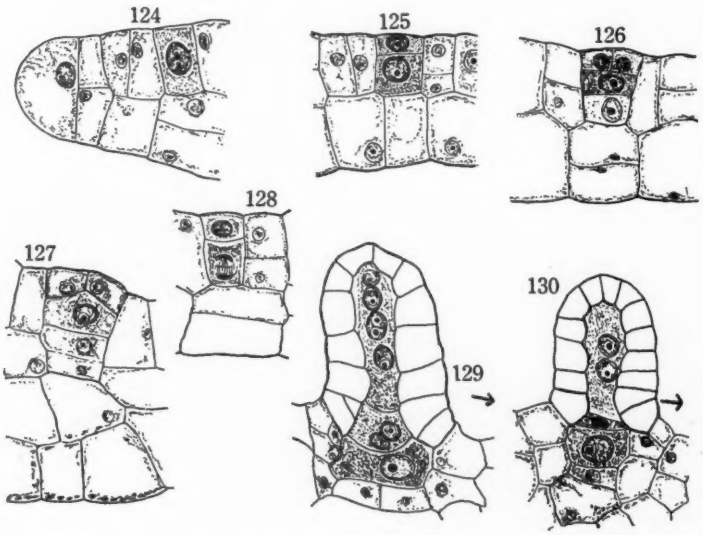


Figs. 114–123.—*Thyrsopteris elegans*, ×300.

hardly be described in terms of the antheridia of the Cyatheae or of the Polypodiaceae. They are similar to some of the largest and most irregular of the antheridia of *Lophosoria*, or to those of the Osmundaceae. A basal cell can usually be distinguished, but there may be two or even three basal cells. The opercular cell, thrown off at maturity, is perfectly definite (fig. 121). In the majority of antheridia, however, it is hardly possible to designate cells as funnel, ring, or primary cover cells. Even when the antheridium apparently consists of five cells of the cyatheoid type it may be large and asymmetrical, and it is difficult to be sure of the number of cells.

This type, when it exists, is doubtless to be looked upon as a reduction form rather than as the characteristic type. The antheridia apparently are formed by a series of wedge-shaped cells. While many antheridia appear to have a primary cap cell which divides twice (fig. 120), there are many which cannot be interpreted in this way (fig. 122).

ARCHEGONIUM.—The archegonium of *Thyrsopteris* appears at about the same stage of development of the thallus as in the Cya-



FIGS. 124–130.—*Thyrsopteris elegans*, ×300.

theae. Figs. 124–128 show sections of the thallus in which the relation of the initial and the young archegonium to the thickness of the thallus can be noted. There is usually only one basal cell (fig. 126) but two are formed occasionally (fig. 127). The neck of the archegonium is longer, as a rule, than those of the Cyatheae; it may be straight but is usually more or less recurved or curved to the side. The same type of modification of the contents and walls of the neck cells occurs as has been described for the Cyatheae. Archegonia with four neck canal cells are not infrequent; in the archegonium

shown in fig. 129 the nucleus of the ventral canal cell had also divided. The position of the wall in the first division of the embryo was not observed.

Thyrsopteris shows a strong tendency to form apogamous growths. This probably accounts for the difficulty experienced in raising sporelings of this species. Mr. BOODLE wrote to me several years

FIG. 131.—*Thyrsopteris elegans*, sporeling, ×1 (photograph by A. S. KINNEY).

ago that they had been unsuccessful at Kew. Although working with *Thyrsopteris* for several years, I have succeeded in raising only one sporeling which has progressed far enough to give hopes of raising it to maturity (fig. 131). The apogamous plants developed vascular structures and the characteristic leaves but did not produce stem or root.

Dicksonieae

The following list gives the eleven species of the Dicksonieae which were investigated and the length of time each was kept in culture.

Culcita macrocarpa[2] Presl. = *Balantium culcita* (L'Hérit.) Klf. 2 and a half years

Cibotium barometz (L.) J. Sm. 5 years

Cibotium chamissoi Klf. 2 and a half years

Cibotium glaucum (Sm.) Hk. & Arn. 7 months

Cibotium menziesii Hk. 2 and a half years

Cibotium regale Linden 1 year

Cibotium schiedii Schlecht. & Cham. 7 months, 8 months, 1 year

Dicksonia antarctica Lab. 6 months, 8 months, 2 years

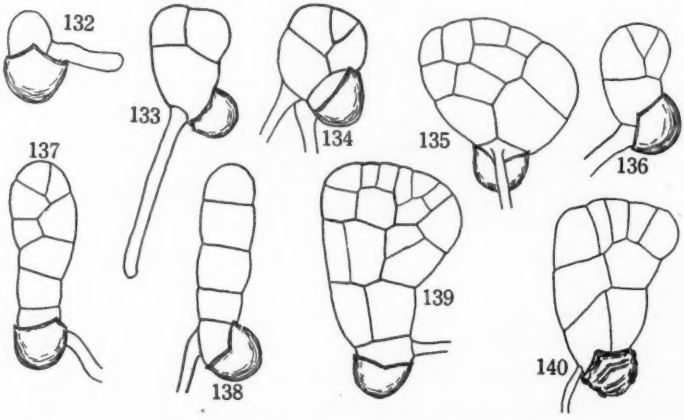
Dicksonia fibrosa Col. 15 months

Dicksonia squarrosa (Forst.) Sw. 8 months

Dicksonia youngiae C. Moore 9 months

VEGETATIVE STRUCTURE

Germination and the early stages of the prothallium in this group are shown in figs. 132–140. It will be seen that the same type pre-

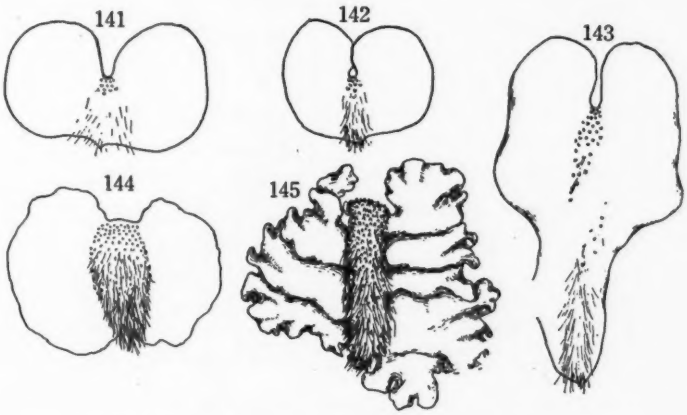


FIGS. 132–140.—Fig. 132, *Cibotium schiedii*, ×165; figs. 133–135, *Dicksonia antarctica;* figs. 133–135, ×165; fig. 134, ×185; fig. 136, *D. fibrosa*, ×165; figs. 137–139, *C. regale*, ×165; fig. 140, *C. barometz*, ×150.

vails here that was found in the Cyatheae and *Thyrsopteris*. The young prothallium passes quickly into the plate stage, leaving at

[2] In the name of this species I have followed MAXON (19) instead of the nomenclature of CHRISTENSEN.

the base in most cases only one cell in which there is no longitudinal division. The tendency to form a filament is very slight in most species, even in crowded cultures. Fig. 140 shows a prothallium of *Cibotium barometz* taken from a clump of 25–30 young prothallia; in the whole clump there were only three prothallia which had more than one cell at the base without longitudinal divisions. The tendency to form a filament is somewhat stronger in *C. regale*, as prothallia of the type shown in figs. 137 and 138 are not uncommon,



FIGS. 141–145.—Fig. 141, *Dicksonia fibrosa*, ×5; figs. 142, 145, *Cibotium schiedii;* fig. 142, ×5; fig. 145, ×2.5; fig. 143, *C. menziesii*, ×6; fig. 144, *Culcita macrocarpa*, ×2.5.

but most of the cells in such filaments divide longitudinally or obliquely after a time (fig. 139).

The prothallia of *Cibotium* and *Dicksonia* when 2–3 months old as a rule are more like those of the polypods than those of the Cyatheae or *Thyrsopteris* (figs. 141, 142); the prothallia are distinctly broad and short with considerable wing development. If fertilization does not take place and the prothallia are kept under favorable conditions the rib develops more heavily, and the prothallia become more massive than any of the Cyatheae which were studied. If the cultures are somewhat crowded the prothallia elongate, but when given an opportunity they broaden rather rapidly

(fig. 143). The rib may become heavy and cause the wings to be lifted; or the wings may grow luxuriantly, producing the curly ap-

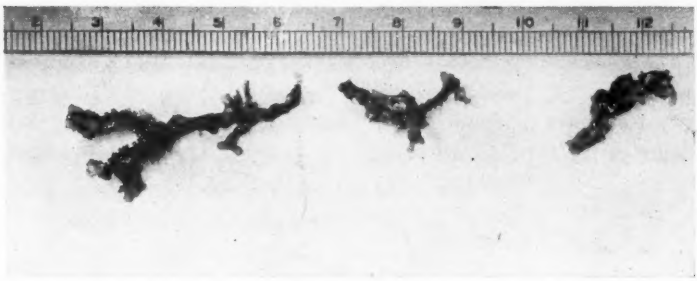
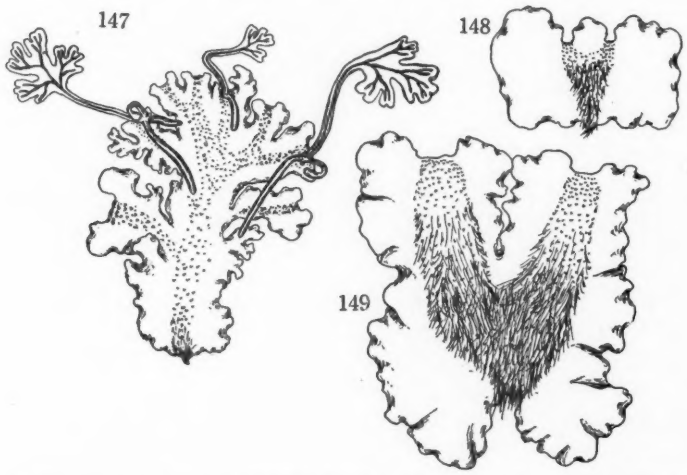


FIG. 146.—*Cibotium barometz;* prothallia four years old, ×1.25 (photograph by A. S. KINNEY).

pearance shown by *Cibotium schiedii* (fig. 145). Forking of the meristem with the production of one or more archegonium-bearing



FIGS. 147–149.—Fig. 147, *Cibotium barometz*, ×2.5; figs. 148, 149, *Dicksonia antarctica*, ×2.5.

branches occurred in *Culcita macrocarpa*, *Cibotium barometz*, *C. chamissoi*, *C. schiedii*, *Dicksonia antarctica*, and *D. fibrosa*. The

prothallia of *C. barometz*, although not exceptionally thick or broad, elongated rapidly and forked freely. The prothallia shown in fig. 146 were from a 4-year old culture. As shown by fig. 147, each branch of the prothallium may act as a unit in the production of sporelings. Forking of the meristem occurred at an earlier stage in *D. antarctica;* the branches were heavier, as a rule, and did not elongate to the same extent. The cushion of such a prothallium as that shown in fig. 149 may become 12–14 cells thick.

No hairs of the type characteristic of the Cyatheae were found on the prothallia of any species of the Dicksonieae included in this investigation. No hairs of any type were found except in the case of a single prothallium of *C. barometz* which bore the structure shown in fig. 150*a*, which is apparently a cylindrical process which may be looked upon as apogamous. These hairs are like those on the young petiole of *C. barometz*, and are quite unlike the hairs of the Cyatheae either in form or origin. This species has been found to produce apogamous growths, and while this process is not definitely sporophytic it shows a wide departure from any gametophytic structure.

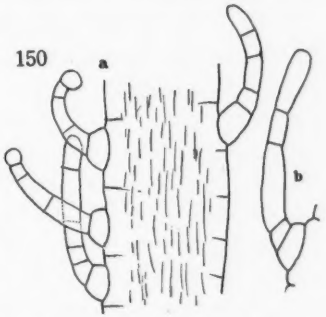


FIG. 150.—*a*, *b*, *Cibotium barometz*, ×105.

BAUKE (1) states that in none of the prothallia of the Polypodiaceae which he studied did he find bristle-like hairs, but that he found them in all of the Cyatheaceae. This would imply their presence in *D. antarctica* and *C. schiedii*, but these two species are among the forms which he expressly states were less fully studied than *Cyathea medullaris*, *H. spectabilis*, and *A. australis*, chiefly owing to lack of material. HEIM (13) reported hairs for *D. antarctica* (*Balantium antarcticum*), and described them as occurring not only on the dorsal and ventral surfaces but also on the margin, but he gave no figures showing the type of hair. I am unable to agree with the accounts of BAUKE and HEIM with regard to the presence of hairs on *D. antarctica* and *C. schiedii*. Many prothallia of both species have

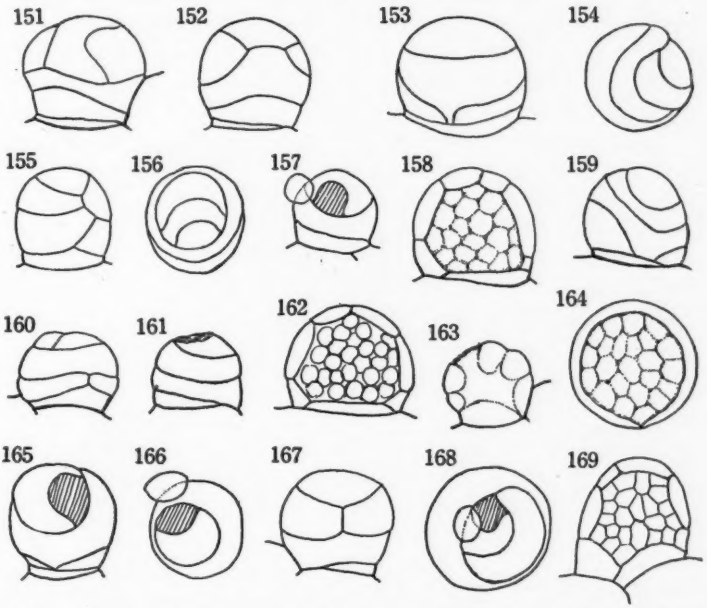
been examined but not a single hair either unicellular or multicellular has been seen. In the case of *D. antarctica* three sets of cultures were carried, two from spores obtained from Fairmount Park, Philadelphia, and one from spores from Kew. The three sets of cultures were similar in habit and range of type. The Kew cultures were kept for over two years. The prothallia were vigorous and grew to a large size for that species (15 mm. in length), many cases of branching occurred, archegonia were produced in great numbers (300–400 on a prothallium). This indicates sufficient maturity for the appearance of much belated hairs but none appeared.

Reproductive structures

The prothallia of the Dicksonieae are monoecious with a rather more pronounced tendency toward dioecism than was seen in the Cyatheae. Many prothallia passed at once to the production of archegonia without apparently any antheridial period. If the prothallium went through an antheridial period the production of antheridia ceased as soon as archegonium production was initiated. Antheridia were usually produced most abundantly on special male prothallia. In most cases these were flat plates with a weak meristem which might persist for many months. In some cases ameristic male prothallia were formed but they were usually more or less elongated plates, rarely filaments. The formation of ameristic filaments is much less frequent in the Dicksonieae than in the Cyatheae.

Antheridium.—The antheridia of the eleven species of the Dicksonieae which were studied are, with the exception of those of *Culcita macrocarpa*, of the same general type as those of *Alsophila*, *Hemitelia*, and *Cyathea*, but are usually somewhat larger (figs. 158, 162–164, 165). They may show from fifteen to thirty-eight sperms in median section, but the usual number is between seventeen and twenty-five (figs. 158, 162, 164). The tendency for the primary cap cell to divide twice (figs. 156, 168) is somewhat stronger than in the Cyatheae, and there are fewer cases of undivided cap cells. The antheridium of *Culcita macrocarpa* is interesting because of its suggested primitiveness. While not larger than others in this subfamily, it is decidedly more irregular and asymmetrical (figs. 151–155). The number of cells in the wall is regularly five but frequently

six. The walls of the cell are oblique and often suggest wedge-shaped cells formed from an apical cell rather than basal, funnel, and ring cells. In many cases the antheridium when viewed from the top appears to be crossed by walls more or less parallel (fig. 154), and

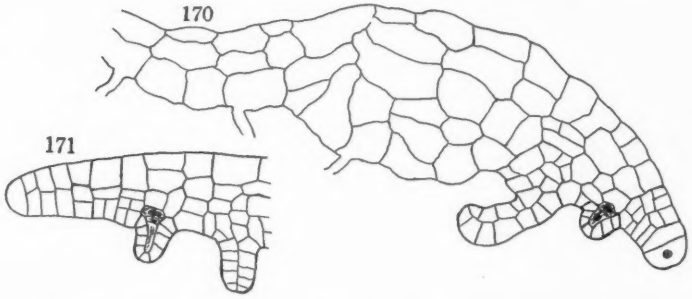


FIGS. 151–169.—Figs. 151–155, *Culcita macrocarpa;* figs. 156, 157, *Dicksonia antarctica;* fig. 158, *D. fibrosa;* fig. 159, *D. squarrosa;* figs. 160–163, *Cibotium chamissoi;* fig. 164, *C. regale;* figs. 165, 166, *C. barometz;* fig. 167, *C. schiedii;* figs. 168, 169, *C. menziesii;* ×300.

there is nothing to suggest a primary cover cell such as is seen in figs. 156, 166, and 168. In the case of the antheridium of *Culcita macrocarpa* it is somewhat difficult to use the terminology which applies to the typical antheridium of *Dicksonia* and *Cibotium*.

ARCHEGONIUM.—In the Dicksonieae archegonial formation begins when the thallus is at the same stage as that noted in the Cyatheae and *Thyrsopteris*, or even later (figs. 170–172). Figs. 170 and 171 show sections of small prothallia; the one in fig. 170 had six arche-

gonia and that in fig. 171 had ten. Fig. 172 is taken from a prothallium of *Cibotium barometz* which had no mature archegonia and one but slightly older than the one shown; the thallus had already attained a thickness of four cells. Cases of two superimposed basal cells are much less frequent than in the Cyatheae, but the rather frequent occurrence of a vertical division in the basal cell of *Culcita macrocarpa* and *Cibotium barometz* (figs. 174, 177), and its occasional occurrence in other species, suggest that the second basal cell in the superimposed type (fig. 178) is formed by a division of the primary basal cell rather than by a division of the central cell. The appear-

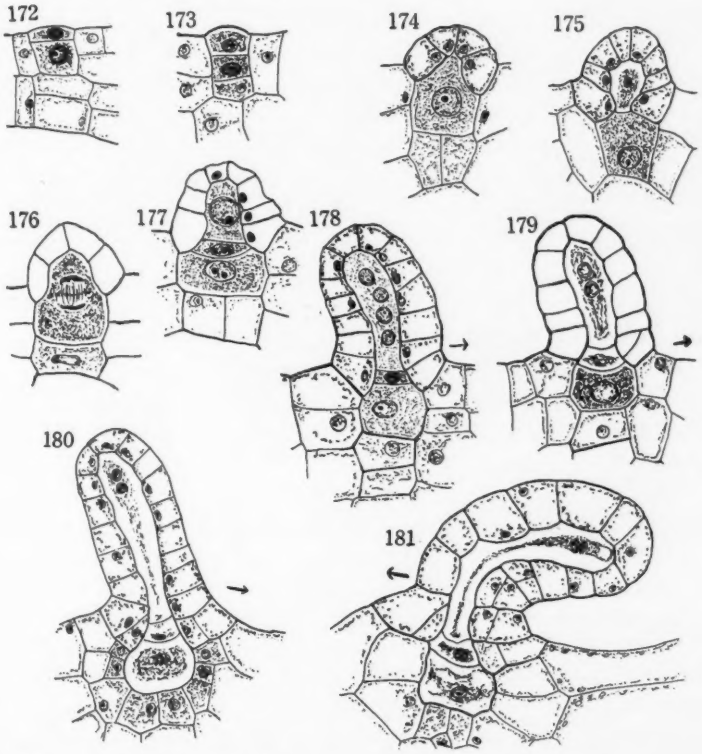


FIGS. 170, 171.—Fig. 170, *Cibotium schiedii*, ×105; fig. 171, *Dicksonia antarctica*, ×105.

ance of the contents of the cells does not indicate that this is a premature appearance of the divisions leading to the formation of the nutritive jacket of the egg such as is shown in fig. 180. Fig. 175 shows a young archegonium which has no basal cell; this is the only case which was found.

The neck of the archegonium in the Dicksonieae is decidedly longer than in the Polypodiaceae and usually longer than in the Cyatheae; it is most like that of *Thyrsopteris*. The neck is usually seven or eight cells long on the anterior side at maturity (figs. 178, 181), rarely six (fig. 179), and occasionally nine or even ten (fig. 180). The tendency to curve away from the notch is more pronounced than in the Cyatheae, especially in *Cibotium* which on the whole has longer necks than *Dicksonia*. The same type of modification of contents and wall of the neck cells which was noted in the

Cyatheae and *Thyrsopteris* is found in the Dicksonieae. Archegonia with four neck canal nuclei (fig. 178) were found in all species included in this investigation.



FIGS. 172–181.—Fig. 172, *Cibotium barometz*, ×300; figs. 173, 179, 180, *C. regale;* figs. 173, 179, ×300; fig. 180, ×245; fig. 174, *Culcita macrocarpa*, ×300; figs. 175, 176, 178, *Dicksonia antarctica*, ×300; fig. 177, *D. squarrosa;* fig. 181, *Cibotium schiedii*, ×300.

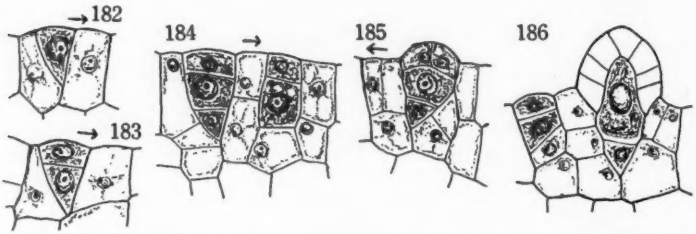
The formation of archegonia from wedge-shaped initials is found more frequently in this group than in the Cyatheae (figs. 182–186). It was found most frequently in *Cibotium barometz* but it occurs not infrequently in *C. regale* and other species in this group.

The first wall of the embryo is approximately in a lengthwise plane of the archegonium.

In addition to the case of apogamous growths reported for *D. squarrosa* in an earlier paper (**27**), apogamy was found in *C. barometz* and *C. schiedii*.

Discussion

The points on which this investigation should shed light are whether or not the group is a natural one, and the connections both downward and upward shown by the group as a whole or by its members. HORVAT (**14**) has given a discussion of the significance of the gametophyte of the ferns in phylogeny with full references to the literature on the subject. The lines of evidence presented by the gametophyte which have commended themselves most strongly



FIGS. 182–186.—Figs. 182, 183, 185, 186, *Cibotium barometz;* fig. 184, *C. regale;* ×300.

to investigators are the structure of the antheridium and the character of the hairs, if any are present. Germination characters and the habit of the mature prothallium, owing to their variability, and archegonial characters, because of their uniformity, have been rated less highly.

In the structure and development of the antheridium there is some uniformity, except for *Lophosoria* and *Thyrsopteris; Culcita macrocarpa* may also be given as not conforming to the type. *Thyrsopteris*, which stands out most sharply as a primitive type in antheridial structure, is also set apart by its sporophytic structure. These three genera are obviously not closely related to one another; it will hardly be questioned that *Lophosoria* is more closely related to *Alsophila* than it is to *Thyrsopteris* or *Culcita*, and that *Culcita* is related to *Cibotium* and *Dicksonia* rather than to *Lophosoria* and *Thyrsopteris*. The antheridia of these three forms are similar in their

irregularity and lack of symmetry; those of *Lophosoria* and *Thyrsopteris* also stand apart in the matter of size. They agree in being suggestive of a primitive type such as that of *Osmunda* or *Todea*, rather than that of the Polypodiaceae. The similarity in type of the antheridium of *Alsophila*, *Hemitelia*, and *Cyathea* to those of *Cibotium* and *Dicksonia* can be explained as due to a reduction from the type of the Osmundaceae which has reached the same stage in both lines. All fern antheridia may be derived from a type formed by an apical cell, as suggested by STEPHENSON (**25**) and shown diagrammatically by GOEBEL (**11**). In the process of reduction and simplification the same type may be reached in several lines. The antheridium of the three subfamilies may be derived readily from the *Osmunda* type. Those of *Gleichenia* are intermediate or at the *Thyrsopteris* stage; in the Hymenophyllaceae and the Schizaeaceae, apparently, reduction and simplification have gone further. So far as the evidence from the antheridia is concerned there is nothing to indicate that the Cyatheae, Thyrsopterideae, and the Dicksonieae have a common ancestor nearer than the Osmundaceae.

The multicellular hairs found on the prothallia of *Lophosoria*, *Alsophila*, *Hemitelia*, and *Cyathea* place the group of the Cyatheae sharply in contrast to the Dicksonieae and *Thyrsopteris*. The type of hair is peculiar in its origin, structure, and distribution. It can hardly be related to any other type except those found on species of *Gleichenia* and perhaps those of *Loxsoma*. In origin and distribution the hairs of the Cyatheae are like those of *Gleichenia*. The hairs of *Gleichenia* differ in being only 2–3 cells long and ending usually in a somewhat swollen tannin-filled tip, while those of the Cyatheae may be 20–30 cells long and 8–10 wide, never ending in a glandular tip. The similarity in mode of origin and distribution is certainly indicative of a genetic connection between the Gleicheniaceae and the Cyatheae. The comparison which has frequently been made since the appearance of the work of SCHLUMBERGER (**23**) of the hairs of the Cyatheaceae to those of *Woodsia* and *Diacalpe* seems not to be significant; the difference in origin and distribution opposes rather than favors a genetic connection. The rare and belated appearance of hairs in *Lophosoria quadripinnata* emphasizes the separation between *L. quadripinnata* and *Alsophila*. The evi-

dence from the hairs is that the Cyatheae are rather closly related to the Gleicheniaceae and probably to *Loxsoma*, but that the connection with the Dicksonieae and *Thyrsopteris* is remote.

As for the question of germination, it is uncertain what value is to be placed on it owing to the plasticity of the prothallium at this stage. In general the prothallia of the Cyatheaceae are less plastic than those of the Polypodiaceae at this stage; the habit of the prothallium in the early stages sets them somewhat apart from the Polypodiaceae but does not definitely ally them with any lower forms. The type which is characteristic of the three subfamilies is distinctly different from that of the Osmundaceae; it has been found in *Gleichenia* spp. and in species of the Schizaeaceae. At present there is probably not enough known about germination types in the lower families and the degree of plasticity which exists to enable valid deductions to be made.

As to the mature prothallium, one character which is more primitive than the polypods is their later maturation, the later attainment of the antheridial and the archegonial stages. Perhaps the tendency of the apical region to fork and produce branching prothallia should be mentioned; and it might be noted that the polypods do not ordinarily develop so thick a thallus, at least in prothallia 8–12 weeks old, as is found in the Cyatheae, Dicksonieae, and *Thyrsopteris*. While in long-continued cultures, as MOTTIER (20) has shown, large forking prothallia may be produced by polypods (for example, *Matteuccia nodulosa*) as well as by more primitive ferns such as *Osmunda regalis*, forking is not initiated at as early a stage as in *Hemitelia* and *Dicksonia*. The tendency to produce filamentous ameristic male prothallia which bear antheridia at an early stage is very much more pronounced in the Polypodiaceae than in the Cyatheae, and it is stronger in the Cyatheae than in the Dicksonieae and *Thyrsopteris*. The early production of antheridia and archegonia increases the probability of early fertilization and sporophyte production. In pushing forward these processes the Polypodiaceae are definitely in advance of the Cyatheae, Dicksonieae, and *Thyrsopteris*, with the Cyatheae approaching them.

The evidence given by the gametophyte stage indicates that the family Cyatheaceae as given by CHRISTENSEN (7) and DIELS (8) is

not a natural group. It gives strong justification for the splitting of the family into two parts, and supports the position taken by BOWER that it should be divided, the main division following the line which he has established on the basis of the origin of the sporangia. It would be too much to say that the evidence from the gametophyte supports the further division of the cyatheoid line and the establishment of the family Protocyatheaceae, but it may be well to point out that the gametophytic characters of *Lophosoria quadripinnata* emphasize the inappropriateness of retaining it in the genus *Alsophila*, and present no difficulty in placing it in a separate family. The gametophyte of *Thyrsopteris elegans* has more in common with those of *Culcita*, *Cibotium*, and *Dicksonia* than with those of any other group, and so far as the evidence from the gametophyte is concerned there is no reason why it should not be included in the Dicksoniaceae.

Summary

1. Under favorable conditions the plate stage follows very quickly after germination; there is ordinarily no filament left at the base of the prothallium. The mature prothallium usually is longer and the cushion heavier than in the Polypodiaceae. Forking of the cushion of the prothallium occurs frequently in old cultures, and in certain species may occur in relatively young prothallia.

2. Multicellular hairs were found to develop more or less abundantly on mature prothallia of all species of *Alsophila*, *Hemitelia*, and *Cyathea*, and to occur rarely on *Lophosoria*. None were found on *Thyrsopteris*, *Culcita*, *Cibotium*, or *Dicksonia*. The multicellular hair arises from a special initial, a wedge-shaped cell cut from the anterior face of a superficial cell near the apical meristem. The hairs are found on both dorsal and ventral surfaces, on or near the cushion, but not on the margin. The hairs may reach a length of 2 mm.

3. The wall of the antheridium typically consists of five cells: an oblique basal cell, a lower ring cell with a "binding wall" on one side; an upper ring cell, an opercular cell, and a crescent-shaped cell formed by the division of the cap cell. Many variations from this type occur. The antheridia of *Lophosoria* are larger and less symmetrical than those of *Alsophila*, *Hemitelia*, and *Cyathea*. Those of

Thyrsopteris are the largest and least symmetrical of the family, ordinarily not conforming to the type just given. The antheridia of the Dicksonieae are usually larger than those of the Cyatheae.

4. The necks of the archegonia in the Cyatheae are straight or slightly curved; they have more cells in the neck and are longer than those of the Polypodiaceae. Those in the Dicksonieae and *Thyrsopteris* are longer than those of the Cyatheae, and are more apt to be recurved. The walls of the neck cells are more or less cutinized. The neck contains two neck canal nuclei not separated by a wall. Archegonia with four neck canal nuclei were found in all species.

5. Apogamous growths were found in *Lophosoria quadripinnata*, *Alsophila excelsa*, *A. armata*, *Hemitelia parvula*, *Cyathea medullaris*, *C. dealbata*, *Thyrsopteris elegans*, *Dicksonia squarrosa*, *Cibotium barometz*, and *C. schiedii*.

6. The evidence from the gametophyte indicates that the family Cyatheaceae, in the broader sense, is not a natural group, but is of polyphyletic origin.

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EVIDENCES OF HYBRIDISM IN SELAGINELLA[1]

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(WITH FIFTY-THREE FIGURES)

Introduction

The genus *Selaginella* represents a survivor of an ancient group whose former supremacy has lapsed toward extinction. The affinities of *Selaginella* are with fossil *Lycopsida* of the Paleozoic and a few other present-day remnants, but that it has achieved diversification among the alien *Pteropsida* is suggested by the large and still increasing number of species described. Over 600 species are now recognized, about 50 of which belong to the primitive and polymorphic "*rupestris* group," which includes all the species of *Selaginella* of the United States with the exception of the eastern ranging *S. selaginoides* and *S. apoda*. Many of the species exhibit a polymorphism which has made the taxonomy of the genus a persistent problem.

The chief purpose of this research was the study of meiosis in the microspores of both native and exotic species of *Selaginella*, to determine whether or not irregularities occur, with the hope of obtaining information on the evolution of species in this extensive genus.

RUSSOW (51), one of the early workers on the vascular cryptogams, did not publish his observations on spore development in *Selaginella* because he considered them defective owing to poor and sickly material. HEINSEN (24) studied the megaspore and female gametophyte, but failed to observe any case of mitotic division either in spores or vegetative tissue, and, as he saw elongated nuclei, he concluded that amitotic division was possible. FITTING (18) investigated megaspore development. He found *S. martensii* unsuited to this purpose because many spores developed anomalously. The study of spore formation in all the species was difficult owing to the smallness of the mother cells and the lack of clear nuclear differentiation. HIERONYMOUS and SADEBECK (28) observed that sterile sporophylls occur in many species; that cones often become vegetative;

[1] Contribution from the Laboratories of Plant Morphology, Harvard University.

that branching of cones and even repeated branching occurs; that in some species megasporangia or microsporangia fail completely; and that vegetative reproduction is common and in some species is provided for by special organs. Especially striking was the constant failure of microspores in all investigated cones of *S. rupestris*, whereas the megasporangia inclosed one or two large megaspores of unusual size with two or three small undeveloped spores. They concluded that some form of apogamy obtains in this species. BOWER (6) referred to the vegetative reversion of cones and to the presence of abortive sporangia at the base of cones in *S. selaginoides* and *S. martensii*. GOEBEL (21) demonstrated that self-fertilization is impossible under normal conditions of sporangial dehiscence.

Miss LYON's (40) study of the developing microspores of *S. apoda* showed that a comparatively small number matured. She states: "Curious aberrations in growth are constantly found. . . . Not more than five-sixths of the potential mother cells divide into spores; the others rapidly disappear." In the summary she states that a large percentage of the microspore mother cells form tetrads, "the largest proportion of which abort at this stage." Only a very small number of sporophytes develop. Local material of *S. rupestris* Miss LYON found almost purely megasporangiate. The megaspore mother cells divided variously: either only two daughter cells were formed or one or both divided again, resulting in two, three or four megaspores; occasionally the mother cell failed to divide and acted directly as a megaspore; also two mother cells might function and form eight megaspores. In any case only one or two spores continued to grow after the early stages. No gametophytic development was seen, and the basal spores differed from the younger ones in size only. Miss LYON considered that this was due to abnormal material until she found sporophytes developing from similar megaspores. It is noteworthy that her figs. 120, 121, and 124 show five "dumbbell" megaspores but no comment was made regarding them. In both species sporophytic development was observed to occur within unshed sporangia. GOEBEL (22) regarded this as an expression of a pathological condition.

Later Miss LYON (41) made a preliminary announcement of apogamy in *S. rupestris*, in a paper which seems to have escaped the

notice of European workers in the genus. She found that embryos are frequently formed from the initial cell of the archegonium, and that until the third or fourth division of this initial it is impossible to determine whether a normal archegonium or an embryo will result.

The only cytological examination of the establishment of spores in *Selaginella* is by DENKE (13). Difficulty in differentiation and the small size of the chromosomes hampered the investigation. All stages of division of the microspore mother cells could be observed in a sporangium whose mother cells had started to divide, and a small percentage of them do not divide. An odd method of spindle formation was observed following the movement of the nucleus to the periphery of the cell. Kinoplasmic threads appeared in the cytoplasm near the nucleus, at first without order; from these delicate threads a bipolar spindle was formed with well defined poles, which continued to grow larger. When the spindle had increased so that its poles almost reached the cell wall, very fine rays were formed from the poles to the nuclear wall, which drew the nucleus (now in prophase) back into the center of the cell onto the spindle. This was the first report of such a spindle formation in plant cells. HERMANN (25) described something comparable in the spermatocytes of the salamander; it has been seen in other animals, and is known as "Hermann's spindle." The heterotypic divisions of the microspore mother cells of the species of *Selaginella* examined were figured as regular; the homeotypic divisions were not studied owing to the smallness of the chromosomes. Attempts to follow the division of the megaspore mother cell were unsuccessful.

MITCHELL (43) and SYKES and STILES (59) discussed the cones of *Selaginella*. In the first paper abnormalities were strongly emphasized; the second pointed out the same conditions: a tendency to reduction of the number of megaspores in various species, frequency of aborted sporangia, and rarity of microsporangia in some species.

BRUCHMANN'S (8) monograph on *S. selaginoides* (L.) Link (*S. spinulosa* A. Br.) noted the low percentage of germination of the megaspores, the small number of archegonia formed, and the absence of development of female prothallia in unshed megaspores. Later (9) he found apogamy in *S. selaginoides* and *S. rubricaulis*. He

was led to investigate the latter because of the very few microsporangia, formed only at the apex of cones sometimes 2 cm. long. All of the megaspores placed on damp blotting paper in a petri dish germinated except very small ones. All embryos were formed behind closed canals with unraised cover cells. There were archegonia in which the canal had opened but the eggs in these never developed; if the cover cells started to arch normally the egg beneath would not form an embryo. In 1919 BRUCHMANN (10) added another apogamous species to those earlier reported, *S. helvetica*, native to Europe, in which practically all archegonial canals remained closed, and beneath those which underwent secondary thickening, embryos developed.

HIERONYMOUS (26) noted two Asiatic species which he suspected of apogamy. Both are widely dispersed in spite of the fact that one (*S. intermedia*) produces no microspores, and in the other (*S. belangeri*) all of the microspores degenerate. Later, working on the taxonomy of material from Australasian islands, he was able to confirm his opinion that many species of *Selaginella* have been able to achieve their otherwise inexplicably wide dispersal through apogamy (27). To species already cited as suspected, he added *S. rugulosa* and *S. longiaristata*. GOEBEL brought back from Brazil *S. anocardia*, a species nearly related to *S. apoda*, which, as it was widely dispersed although practically without microsporangia, he suspected of apogamy, which he was able to demonstrate (23). GOEBEL suggested that apogamy occurs also in *S. apoda*, and that for this reason Miss LYON (40) found megaspores germinating while still in the strobili.

Both BRUCHMANN (9) and GOEBEL (23) believed that there was lack of reduction in the megaspore formation of apogamous *Selaginella* species. This theory was based chiefly on the cytological investigation by STRASBURGER (58) of the reduction divisions of the apogamous *Marsilia drummondii*. This species presents some illuminating parallels to conditions observed in species of *Selaginella:* it was found to be polymorphic to a marked degree; some forms had only sterile microspores; the two accessory canal cells failed to disintegrate and the archegonia remained closed in prothallia capable of developing parthenogenetic embryos, and the spindle was formed in the cytoplasm of the mother cells during prophase. Observations

of meiosis in the megaspore mother cells showed that two kinds of megaspores were developed, as approximately half of them underwent a somatic division, producing megaspores with the diploid number of chromosomes, and about half went through a reduction division which in some cases was regular and in others very irregular. In the microspore mother cells irregularities were so extreme that in most of the forms the first division was never completed. The prothallia developed from unreduced megaspores formed archegonia which maintained closed canals behind which embryo formation took place.

Materials and methods

The material studied consists of six exotic species gathered through two seasons at the greenhouses of the Harvard botanic gardens, and two native species. The greenhouse species are *S. amoena* Hort. (*S. caulescens* (Wall.) Spring) from Asia; *S. emmeliana* Van Geert from Ecuador and Mexico; *S. flabellata* (L.) Spring, universal in tropical and subtropical regions of America, Asia, and Polynesia; *S. kraussiana* (Kunze) A. Br. from Africa; *S. mandaiana* Hort. and *S. martensii* Spring var. *variegata* Hort. Makoy. *S. martensii* is from Mexico. According to HIERONYMOUS and SADEBECK, the variety known as *variegata* is only a sickly garden form with streaked or entirely white portions on some shoots. BAILEY (1) spoke of *S. mandaiana* briefly in a footnote as a recent introduction by W. A. MANDA which cannot be satisfactorily placed. MANDA, a New Jersey seedsman, gave the following information concerning its origin:

> When we used to import orchids from different parts of the tropics, we saved all the refuse and trimmings and planted them carelessly under a bench to see if something might come up of horticultural interest, and this *Selaginella* was one result.

The native species examined are eastern ones, *S. apoda* (L.) Fernald (*S. apus* Spring) and *S. rupestris* (L.) Spring. *S. apoda* came from Core, West Virginia, and Pepperell and Granby, Massachusetts; *S. rupestris* from seven regions: Woburn and three different stations on the Mount Holyoke-Norwottuck Range, Massachusetts; Cape Elizabeth, Maine; Berlin, New Hampshire; Bolton, Vermont; Sandy Cove, Nova Scotia; and the shores of Lake Huron.

For the greenhouse species two fixatives were used, Rabl's 0.75

per cent chromo-acetic acid, and Carnoy's fluid; the native species were killed in Carnoy's only. The use of an exhaust pump aided rapid penetration of the tissues. A softening agent (10 per cent aqueous solution of a saturated solution of sodium chlorate in hydrofluoric acid) was used to overcome resistance encountered in cutting the tough spore coats and sporangial walls. The sporangia were then pricked to allow penetration of the nitrocellulose in which the cones were subsequently imbedded. Sections were cut 5 and 10μ, overstained with Haidenhain's iron-haematoxylin, differentiated, and sometimes counterstained with eosin.

Dividing mother cells occur rarely and great difficulty was experienced in properly differentiating them. Not only are the mother cells surprisingly minute, so that little can be seen under high-power, but their callose coat is of comparatively great thickness, and reflects the light in a water mount to such a degree that usually nothing can be seen but the color of the cytoplasm.

Investigation

Irregularities in development and spore conditions

Some abnormalities in the cones were noted. A cone of *S. emmeliana* had split at the summit into two vegetative shoots; a cone of *S. elegans* had bifurcated, and in *S. martensii* var. *variegata* a bifurcated cone appeared in which one of the branches had redivided. Mother cells with two and three nuclei were seen in *S. apoda* and with two nuclei in the variety of *S. martensii*. In most species a number of microspore mother cells failed to divide; in *S. martensii* var. *variegata* this group amounted to half of the mother cells. In this species and *S. emmeliana* an exine coat was laid down about such cells, some of which already showed signs of disintegration, whereas in *S. apoda* this did not occur, but the mother cells which did not divide disintegrated rather promptly.

In *S. amoena*, *S. martensii* var. *variegata*, and *S. mandaiana* collapsed young sporangia are conspicuous. When the collapse is in process the mother cells may appear healthy or the cytoplasm may show vacuolation; the wall may look normal or signs of degeneration may appear in the dehiscing layer or in the tapetum. In *S. emmeliana* the contents of very young sporangia frequently abort; the wall

maintains its position and the two outer layers look normal but the mother cells collapse and the tapetum has an abnormal staining reaction or is absent.

There is a high percentage of abortion in the microspores of all the species, with the possible exception of *S. kraussiana*, which sheds its sporangia so early that observations of spore conditions were curtailed. In fact *S. apoda* is the only species in which any advanced development of the male gametophyte was seen, and only a few spores of any of the species start gametophytic development. Spores of varying sizes and staining capacities appear; there are collapsed, wizened, and large empty spores with perfectly sculptured exine coats. A brownish exine instead of the natural clear yellow is formed about all of the spores in some sporangia. The presence in abundance of small yellow granules in a viscid medium is frequently observed in sporangia, corresponding exactly in color with normal exine coats, and suggesting an abnormal functioning of the tapetum. Undeveloped spores, discolored exine coats, and yellow granules occur also in the megasporangia.

Spindle formation

The megasporangia and microsporangia are indistinguishable until the mother cells are formed. When the microspore mother cells are established they round up rather promptly, while growth of the sporangial wall continues, so that they lie freely in a fluid-filled cavity at an early stage. All of the non-functioning cells of the megasporangium fail to round up, and show highly vacuolated cytoplasm, contrasting conspicuously with the densely protoplasmic, dark-staining sphere of the megaspore mother cell (figs. 18, 19). This cell stains darker, is slightly larger, and has a thicker callose coat than the microspore mother cells of the same species.

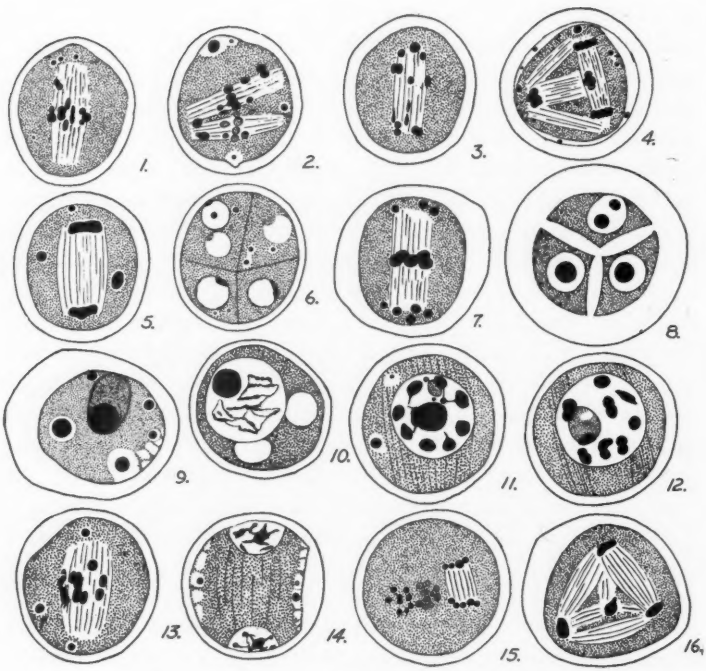
The method of spindle formation in the megaspore and microspore mother cells is the same in all species, although the phenomenon is more prominent in some species than in others. There is no difference caused in its appearance by the two fixatives used. As the nucleus of the mother cell starts into activity a very fine network of chromatin appears, forming the synaptic knot at the outer edge of the nucleus, which by this time has moved to the periphery and ap-

pears to protrude somewhat from the cell. The spindle fibers apparently develop from some of the cytoplasmic contents, and appear as fibrillae stretching in a broad formation across the cell (figs. 25, 26). They lie on the opposite side of the cell from the nucleus; but later the nucleus returns to the center and is enveloped by the spindle fibers (figs. 11, 12). The different views of spindle formation cannot be interpreted so that they correspond with DENKE'S (13) description of spindle poles and a spindle which grows in length. The growth observed is in breadth and no polar points were seen. It may be noted that the Carnoy killing fluid used in this investigation is similar in composition to DENKE'S acetic-alcohol fixative.

REDUCTION DIVISIONS

The critical stages of division of the microspore mother cells are of rare occurrence and are found in sporangia whose cells are predominantly in prophase or in tetrads. The divisions are evidently rapid and the mother cells lacking in concerted action. Because of the smallness of the cells, the presence of bodies taking the chromatin stain (which commonly appear in the cytoplasm) is a confusing factor. Since such bodies may occupy positions which might be assumed by the chromosomes it becomes difficult to judge whether one is dealing with chromosomes or what may be called for convenience "chromatin bodies." In some species the conditions are so extreme that no conclusions can be reached.

1. *S. amoena.*—A clear case of a regular heterotypic metaphase was never seen. Many cells in the first division have chromosomes scattered all over the spindle in such a way that one does not know whether to consider the condition metaphase or anaphase, suggesting FEDERLEY'S (16) term "metanaphase." In other cases there is an alignment of some chromosomes at the equator, however, with others scattered on the spindle (fig. 1). There are some metaphases which at first seem perfectly regular, as a black plate of chromosomes stands out sharply at the equator, but closer inspection shows also chromatin bodies of the same size as the univalent chromosomes at each pole, and it is impossible to be sure whether these are chromosomes which have departed prematurely from the equator, or whether they are other chromatin bodies (fig. 7). The narrowness



FIGS. 1–16.*—Figs. 1–8, *S. amoena*, microspore mother cells: 1, irregular heterotypic metaphase; 2, irregular homeotypic metaphase; 3, irregular heterotypic division with univalents and bivalents in abnormal positions; 4, regular homeotypic telophase with chromatin bodies in cytoplasm; 5, heterotypic telophase with chromatin bodies in cytoplasm; 6, tetrad stage with pseudonuclei, one spore has extra true nucleus; 7, heterotypic metaphase with chromatin bodies or univalent chromosomes at poles; 8, tetrad showing heavy callose coat. Figs. 9–16, *S. apoda*, microspore mother cells: 9, mother cell with large chromatin bodies in cytoplasm (attempt at amitotic division?); 10, mother cell showing clearly defined vacuoles in dense cytoplasm (thought by LYON to be concerned in spindle formation); 11, diakinesis with nine chromosome masses, irregular nucleolus, chromatin threads, and small chromatin bodies (spindle appears enveloping nucleus); 12, diakinesis with nine chromosomes, some clearly bivalents, vacuolating nucleolus, and spindle enveloping nucleus; 13, heterotypic metaphase, slightly irregular, with one unpaired chromosome; 14, interkinesis, cell vacuolated; 15, regular homeotypic telophase; 16, homeotypic telophase showing typical sextuple spindles.

* Drawings made with 1.5 mm., n.A. 1.3 apochromatic Zeiss oil immersion objective and a 10× compensation ocular. A cell was drawn with Bausch and Lomb camera lucida and enlarged twice. All figures drawn to this scale except figs. 17–24, which are on a lower scale. Drawings reduced approximately one-half in photographing.

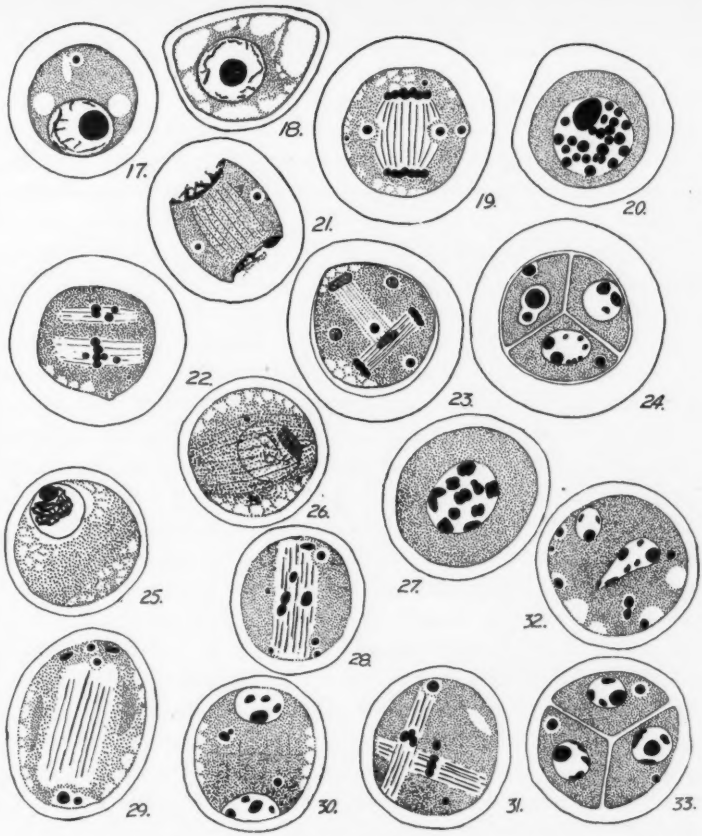
of the plate suggests the former condition. No estimate of the chromosome number could be made. In most of the dividing cells there are varying amounts of chromatin in the cytoplasm, usually near the cell wall. At interkinesis the chromatin bodies appear near the poles or the equatorial plane but always at the periphery of the cell, and a clear zone in the cytoplasm is seen about them; this halo is usually present at all stages but seems especially pronounced at interkinesis and the tetrad stage (figs. 1, 2, 4–6). In fig. 4, at homeotypic anaphase, chromatin bodies are shown lying outside the cytoplasm but within the callose coat; this is comparable with cells noted in *S. kraussiana* (figs. 34, 36), where there is evidently an effort to eject chromatin bodies from the cytoplasm. The homeotypic division is sometimes regular and sometimes not (figs. 2, 4). At the end of this division there are commonly present in the mother cells four nuclei and a number of pseudo-nuclei. However, some cells have only two or three large nuclei; one was observed with one nucleus of normal size and twelve small nuclei, and one had five nuclei of equal size and similar organization besides a number of pseudo-nuclei (fig. 6). In the last case walls had already been laid down, cutting off four spores, which resulted in polycary in one of the spores. No case of polyspory was observed in *Selaginella*, and so far as the writer knows, the formation of more than four microspores in the mother cells of pteridophytes has never been reported, even in forms where extreme irregularities occur during meiosis (31).

2. *S. apoda.*—In this species the chromosomes at diakinesis are commonly distributed about the periphery of the nucleus as well defined entities, and often the bivalency of some of the chromosomes is apparent. In fig. 12 such a condition is shown, with nine bodies, five of which are manifestly bivalent. The nucleolus is vacuolating as is usually the case in late diakinesis. The cell shown in fig. 11 is evidently at an earlier stage, as the nucleolus is budding off chromatin, and three small chromatin bodies appear connected to the chromosomes by delicate chromatic threads. Here again nine masses appear. As some deviations from this number were observed, and as there is reason to doubt the constancy of bivalent formation (since apparently unpaired chromosomes have been seen at diakinesis and metaphase), the writer hesitates to assign a definite chromosome

number to *S. apoda*. The heterotypic division generally shows a slightly irregular metaphase and early anaphase, and sometimes the presence of an unpaired chromosome is evident. While chromatin in the cytoplasm is not a constant feature in this species, it is usually present (figs. 11, 13, 14). Many cells in division and at interkinesis are much vacuolated (fig. 14). The homeotypic division is regular. At the telophase of the second division four additional spindles appear, connecting the four poles of the two division spindles (fig. 16). This appearance is characteristic in *Selaginella*.

Fig. 9 presents an odd condition found in one sporangium. The cell drawn is the most illuminating for the interpretation of the situation, as the original nucleus is readily distinguished and it is evident that the nuclear-like bodies consist of chromatin budded off from the nucleus, each of which has formed a clear space about itself in the cytoplasm. In most of the cells the nucleus has lost more of its chromatin, and five or six seeming nuclei appear; however, one of these is always larger than the others although it does not always show a different organization. Without careful study of all the cells it might be assumed that the meiotic divisions had occurred with irregularities which had resulted in supernumerary and unequal nuclei. In one cell of this sporangium a division spindle appears.

3. *S. martensii*.—Diakinesis is often clear, although sometimes many of the chromosomes are massed against the nucleolus. Only occasionally can bivalency be seen definitely, and probably a considerable number of the chromosomes are univalents. In the cell shown in fig. 20 twenty-four chromatic bodies appear, which is the highest number counted; these all appear to be univalents. In many mother cells chromatin is seen in the cytoplasm before the prophase starts, but the amount is much greater at the anaphase and at interkinesis, when chromatin bodies regularly occupy peripheral positions in the equatorial plane and appear like small nuclei (figs. 17, 19, 21, 23). Metaphases and early anaphases are rarely seen, and no irregularities were observed except in the cell shown in fig. 22, which may be either a heterotypic metaphase with a split in the spindle, or a homeotypic with a difference in size of the spindles caused by a difference in chromosome number. An outstanding feature in this variety is the extreme vacuolation apparent in many cells, especially



FIGS. 17–33.—Figs. 17–24, *S. martensii* var. *variegata* (figs. 18, 19, megaspore mother cells, other figures microspore mother cells): 17, clearly defined vacuoles in dense cytoplasm; 18, degenerating mother cell from megasporangium; 19, late anaphase with chromatin bodies at equatorial periphery (callose coat is comparatively thicker than in microspore mother cells); 20, diakinesis showing twenty-four chromosomes (spindle inadvertently omitted); 21, interkinesis showing polar region vacuolate and chromatin bodies at equatorial periphery; 22, metaphase, either heterotypic with split spindle or homeotypic with spindles of unequal size; 23, homeotypic telophase with chromatin bodies at equators; 24, tetrad with pseudonuclei. Fig. 25, *S. amoena*, microspore mother cell: early stage of spindle formation. Figs. 26–33, *S. emmeliana*, microspore mother cells (except fig. 27, which is megaspore mother cell): 26, early stage of spindle formation viewed from point opposite nucleus; 27, diakinesis showing ten chromatic bodies, no nucleolus distinguishable, spindle inadvertently omitted; 28, irregular heterotypic spindle showing chromatin bodies at poles; 29, vacuolated cell at interkinesis showing failure of nuclear formation at one pole; dark bodies parallel to spindle apparently chromatin being absorbed by cytoplasm; 30, interkinesis with chromatin bodies in range of spindle (laggard chromosomes?); 31, homeotypic metaphase with chromatin in cytoplasm; 32, cell at end of second division, probably resulting from one like that in fig. 29; 33, tetrad with pseudonuclei.

at the poles during anaphase and interkinesis (fig. 21). The pseudonuclei so prominent at division stages (figs. 19, 21, 23) are often seen within young daughter cells (fig. 24); but they disappear quickly, either by absorption into the cytoplasm or by being taken up by the nucleus.

4. *S. emmeliana.*—At diakinesis the nuclear volume does not enlarge appreciably; the chromosomes are closely crowded together, and often there are small particles of chromatin present, so that attempts to count the chromosomes are unsatisfactory. If there is a nucleolus present it must be very similar in size to the chromosomes. Fig. 27 shows diakinesis in a megaspore mother cell which is somewhat bigger than the microspore mother cells. The bivalency of most of the chromosomes is apparent, but whether one of the ten masses is a nucleolus is doubtful. DENKE gives the chromosome number in *S. emmeliana* as eight.

Usually chromatin bodies are seen in the cytoplasm before prophase begins, and increase continually until at the homeotypic division there is a greater bulk of basic-staining substance in the cytoplasm than there is on the spindle. The cells during division stages assume a muddy appearance, and the spindle fibers take the stain deeply, especially at the second division when sextuple spindles appear, so that differentiation which brings out the chromatin bodies fails to reveal chromosomes on the spindle fibers. Often at telophase and interkinesis there seem to be fine bodies on the fibers. Fig. 28 shows a heterotypic division characteristic of the few seen. One wonders why so few chromosomes appear in these cells and similar ones in other species (*S. kraussiana*, fig. 34). The simplest explanation is that some or all of the chromatin bodies in the cytoplasm and near the poles of the spindle are chromosomes which have been ejected from the spindle. Another possibility is that some of the chromosomes disintegrate into fine bodies which become strung out on the spindle fibers and so cause them to stain darkly. If these gradually become absorbed by the cytoplasm, the dark muddy appearance characteristic of the division stages would be explained.

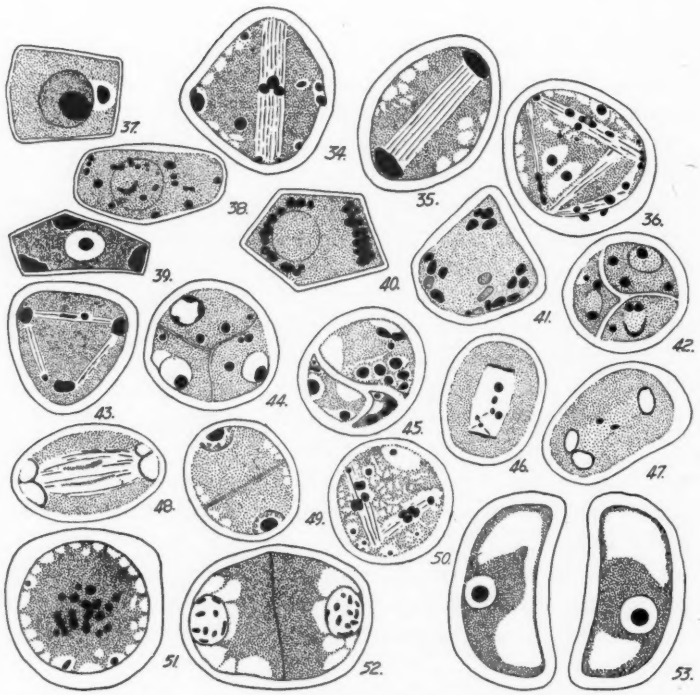
At interkinesis, although there are sometimes two daughter nuclei without any chromatin elsewhere in the cell, the condition shown in

fig. 30 is more common. The small nuclei, instead of being at the periphery of the cell and largely at the equatorial plane (as in *S. martensii* var. *variegata*), are in the plane and range of the spindle, which suggests that they are formed from laggard chromosomes remaining on the spindle. Fig. 29 shows that a daughter nucleus has failed to form at one pole, and its place is taken by a number of small nuclei. A variation of this condition, in that two nuclei are formed at one pole at interkinesis (neither occupying the central position), is frequently seen. The cell shown in fig. 32 may be the product of such a condition. Explanation of these observations is hampered by the minuteness of the cells and the scarcity of earlier stages. Fig. 29 depicts a condition seen a few times in *S. amoena* and commonly in this species. Elongated bodies, staining grayish blue with haematoxylin, appear in the cytoplasm on each side of and parallel to the spindle in heterotypic telophase and interkinesis. These bodies are probably chromatin which is disintegrating in the cytoplasm. Small nuclei are seen in the tetrad cells; it is believed that at least some are formed by chromosomes.

5. *S. flabellata.*—The mother cells in this species were the smallest seen and the chromosome number seems to be low. A number of divisions were observed, but the extreme size of the figures makes it more impossible than in the other species to determine whether the apparent laggards are really chromosomes or are other chromatin bodies. A number of heterotypic metaphases with neat plates had distinct chromatin bodies of the size of the chromosomes at the poles, very much like the cells in *S. amoena* (fig. 7).

6. *S. mandaiana.*—This species of uncertain origin bears cones in the great profusion characterizing all of the species studied except *S. kraussiana*. Large quantities of the cones abort all their sporangia while in the mother cell stage, however, so that they are visibly empty. Thousands of mother cells were seen in this species in extremely anomalous stages, which can be assigned to two different modes of amitotic division. The more prevalent and remarkable one will be described first.

In this form of division the contents of the nucleus pass out into the cytoplasm, the cell divides somewhat quantitatively, and new nuclei are organized (figs. 37–45). Nuclear activity begins while the



FIGS. 34–53.—Figs. 34–36, *S. kraussiana*, microspore mother cells: 34, heterotypic metaphase showing evident effort to eject some chromatin from cytoplasm; 35, heterotypic telophase with vacuolated cytoplasm; 36, erratic division with malformed spindles, vacuolated cytoplasm, and many chromatin bodies, some ejected from cell. Figs. 37–50, *S. mandaiana*, microspore mother cells: 37, chromatin ejected from cytoplasm formed into large mass with halo, nucleus clearly defined; 38, chromatin in cytoplasm in small masses, some passing through nuclear membrane; nucleoplasm not distinguishable; 39, chromatin massed at three points, nucleolus and nucleoplasm well defined; 40, no chromatin left in nucleus although nuclear membrane persists; 41, chromatin bodies collecting in different peripheral regions, no nucleus; 42, tetrad without sharp 120° angle characteristic of trifacial spores of *Selaginella;* 43, sporadic cell with malformed spindle occurring among mother cells dividing as shown in figs. 37–42; 44, tetrad lacking sharp 120° angle; 45, tetrad walls laid down, nuclei not yet organized; 46, nucleus elongate, chromatin at poles (seen as ring in oblique views) and chromatin masses and chromatin threads toward center; 47, later stage, nucleus more elongate, one polar ring split into two and nucleoplasm hardly distinguishable; 48, dark bodies on spindle interpreted as degenerating chromatin; 49, interkinesis, wall forming; 50, irregular heterotypic division, impoverished cytoplasm. Figs. 51–53, *S. rupestris*, megaspore mother cells: 51, polar view of heterotypic metaphase; 52, wall laid down at end of first division; cytoplasm vacuolate at poles; 53, dyad megaspores.

mother cells still lie closely together in the unexpanded sporangia. This is in striking contrast to normal early meiosis in *Selaginella*, when the rounded mother cells lie freely in the cavity of the enlarged sporangia. Fig. 37 shows a cell with much chromatin extruded into the cytoplasm as a formless mass, the nucleus still retaining chromatin sufficient to distinguish the nucleoplasm from the cytoplasm. In fig. 38 passage of chromatin from the nucleus to the cytoplasm is seen; there is less chromatin in the nucleus than in the cytoplasm, and there is no distinction in the reaction of the two plasmas to the stains. On the other hand, in fig. 39 a cell appears which still shows nucleoplasm although most of the chromatin is in the cytoplasm, massed in three places as distant from one another as possible. In fig. 40 no chromatin is left within the nucleus, but the nuclear membrane is still clear; in the next figure the membrane is gone and the chromatin bodies appear congregated at different points. This chromatin gives no suggestion of being organized as chromosomes. One of the surprising features in these cells is the great quantity of chromatin, apparently far exceeding the volume present in the chromosomes of the normal division. This may be due to differences in physical condition. Fig. 43 shows an erratic spindle formation in a sporangium filled with cells of the general appearance shown in fig. 41. When sporangia were first seen, filled exclusively with cells in the various stages described, it was thought that they were going through a disintegration process and would immediately degenerate. Examination of thousands of sections, however, shows that if this process occurs in a cone all of the subsequently formed sporangia divide their mother cells in the same way, and that abortion of the cells does not occur, at least not until after tetrads have been formed. Figs. 42, 44, and 45 depict tetrads from such cones, all showing a failure of the walls to meet in the sharp 120° angle characterizing the spores of *Selaginella* which are consistently trifacial (figs. 8, 24). The nucleoplasm of two of the nuclei of the tetrad shown in fig. 42 is only weakly distinguished from the cytoplasm by the stain, whereas in the third nucleus there is practically no distinction. Fig. 45 probably is an early stage in the evolution of nuclei from the scattered chromatin, as the focal point of the future nucleus can be recognized in only one cell. The most advanced stage shown is fig. 44

but in this there is still chromatin in the cytoplasm which must be destined either to incorporation in the nucleus or absorption by the cytoplasm. Mother cells in *S. apoda* (fig. 9) seem to have passed through a comparable extrusion and division of chromatin, instead of undergoing the ordinary reduction division.

In cones showing less anomalous conditions the usual early spindle formation was not seen, and the only sign of ordinary prophase was limited to one sporangium. In sporangia showing the second form of amitotic division, about half the cells are in a curious state which suggests both diakinesis and anaphase (fig. 46). The nucleus is elongate and appears somewhat oblong. In this, and in the absence of a nucleolus, the presence of chromatic threads and of dark bodies at the ends of the nucleus, it differs from diakinesis. In some cases the sides of the nucleus and the nucleoplasm are not so clear as in others. In some the dark bodies at the ends are seen to be circular, and are interpreted as aggregations of chromatin at the polar edges of the elongated nucleus, which thereby accounts for the comparatively small chromatin content in the heart of the nucleus. This stage persists for a long time, and its development is not easily traced as subsequent stages are rarely observed. Fig. 47 shows the nucleus more elongate, and so invaded by the cytoplasm that it is almost indistinguishable. The ring of chromatin has formed two rings at one of the poles; these inclose a clear area, and it is evident that from each ring a daughter nucleus will be formed. In some cells two rings appear at each pole. Invariably some chromatin seems left behind in this reorganization. The condition shown in fig. 48 would be considered a later stage of that shown in fig. 47 except for the presence of the spindle, which leaves the matter in doubt. The material in the center of the cell, which appeared as a pale bluish mass, is thought to be disintegrating chromatin which was left behind either in the peculiar direct division just described or in an indirect division. These two forms of division are commonly found associated in the same sporangia.

7. *S. rupestris*.—The cones collected in seven widely separated regions show that this species is almost wholly megasporangiate; from one station fifteen cones were cut in which there were only two microsporangia, and those were both in one cone. Miss LYON (40)

states that microspores are formed in the spring and megaspores in the summer, but the writer saw no indication that the occasional microsporangia are formed exclusively in the spring, or even that this is a usual time for their formation.

Because of the rare occurrence of microsporangia there was no opportunity to observe the meiosis of the microspore mother cells, but some divisions in the megaspore mother cells were seen. The functioning megaspore mother cell is densely granular, stains deeply, and is difficult to decolor. In general in *Selaginella* the prophase of the reduction division is a comparatively prolonged stage, as it is commonly encountered. It is of interest, therefore, that no typical prophases were seen in megaspore mother cells of *S. rupestris*. In two cases where the stage was indubitably one of early meiosis, instead of the expected spireme thread an asymmetrical and elongate nucleus showed lumplike masses of chromatin distributed through it. Fig. 51 shows the heterotypic metaphase from polar view. This is not very illuminating except that it reveals a comparatively high chromosome number in this species, which has significant bearing. The next figure is of an interkinesis, if that term can be used when a wall has been laid down; and division must be considered to be completed in the light of the common occurrence in this species of dyads such as are shown in fig. 53. However, many of the megaspore mother cells proceed through both divisions. Counts of the number of megaspores in sixty-eight sporangia taken from material from three different stations gave the following results: 44 per cent contained two megaspores; 28 per cent three megaspores; twenty-five per cent four megaspores, and 3 per cent six megaspores. Occasionally eight megaspores appeared in a sporangium. Regardless of the number of spores, more than two never grow, and in about half of the sporangia only one spore grows. Such large spores are frequently dumb-bell or kidney-shaped, suggesting an incompleted nuclear division which would result in a diploid supply of chromosomes. No gametophytic development was observed, although many are large and old.

The microspores in this species proved to be of great interest. The dumb-bell shape is often encountered, and occasionally bizarre forms. Usually all the spores are full of food and seem to be developing, but advanced stages were not seen. It was continually noticed

that besides being surprisingly large, the spores apparently lay in pairs. To check this, a microsporangium was pricked out in water on a slide where the general occurrence of dyad microspores was unquestionably demonstrated. The spores were commonly tightly associated in pairs, and when they lay separately the markings indicated that two spores had lain together when the first layers of the exine coat were deposited. No triradiate markings were seen, but a few cases were observed where a spore had two or three small collapsed spores clinging to it. As in the sections of imbedded material, the spores were large and elongate, as would result from one division of a mother cell. The significance of dyad formation will be considered in the discussion.

Discussion

Since the days of LINNAEUS, FOCKE (19), and KERNER (35), natural hybrids have been recognized by taxonomists and have provided a subject of slowly growing interest. Doubtless the increased knowledge of natural hybrids is to be correlated with more intensive studies within a group or a definite region. Thus BRAINERD'S (7) work on natural *Viola* hybrids of North America described eighty-two formed among thirty of our seventy-five species. He concluded that hybridization is limited only by failure in cohabitation of species. Hybrids have been found among the pteridophytes, both in nature and in culture. BENEDICT (3) cited fifteen crosses among six native species of *Dryopteris*.

STERILITY OF HYBRIDS.—From the time of KÖLREUTER (36), sterility has been recognized as a conspicuous characteristic of most hybrid forms, although there are hybrids which are fertile. The persistent presence of sterile pollen in a species growing under normal conditions is now generally regarded as indicative of the impurity of the line. HOAR (29), STANDISH (57), and COLE (12) found in pollen sterility indications of widespread hybridism in *Rubus*, *Crataegus*, and *Rosa* respectively, conclusions later verified by the more detailed cytological and genetical observations of PEITERSEN (46), LONGLEY (38, 39), PENLAND (47), BLACKBURN and HARRISON (4), and TÄCKHOLM (60).

REDUCTION DIVISIONS IN HYBRIDS.—Although in the early years of cytological investigation there was an idea current that only

irregular meioses occurred in hybrids, and that the consequent variability in chromosome distribution was the exclusive cause of hybrid sterility, further research has shown that not all hybrids show abnormalities in the reduction divisions. If the parental chromosomes are equal in number the pairing may take place neatly and the divisions proceed with regularity; however, the pollen is usually largely sterile. Irregular distribution of chromosomes at the reduction division has been described for great numbers of hybrids. In some forms all of the chromosomes appear paired at diakinesis, but in most forms which have parents with differing chromosome numbers a definite number of bivalents and of univalents appear. The bivalents divide normally; the univalents may remain undivided in the first division or may split. The classic example of the first type of univalent action is the hybrid between *Drosera longifolia* (n-20) and *D. rotundifolia* (n-10) examined by ROSENBERG (**48**), in which the unpaired chromosomes at the heterotypic division pass without order to either pole, lag behind so that they form small nuclei, or are left in the cytoplasm where they disintegrate. GEERTS (**20**) found the same chromosome action in a cross between *Oenothera lata* (n-7) and *O. gigas* (n-14). SAX'S (**53**) triploid *Triticum* hybrids followed this scheme in meiosis, but in his pentaploid hybrids the univalents split at the heterotypic plate after the division of the bivalents, resulting in spores whose chromosome numbers varied in a comparatively wide range. This last phenomenon also appears in many species of *Rosa*, whose cytology was studied by BLACKBURN and HARRISON and more extensively by TÄCKHOLM.

In extreme cases there is variable pairing of chromosomes or none. FEDERLEY (**16**) raised *Pygaera* hybrids, and found that there was no pairing, and both divisions of the spermatocytes were equational. Most of the cells showed anomalous conditions and "pathological appearances." WOLSEDALEK (**66**) found the cause of sterility in the mule in the destruction of the primary spermatocytes through many forms of degeneration in reduction processes: synizesis was lacking; pairing was very variable; spindle formation was bizarre; giant cells appeared; in some cells of ordinary size the abnormal number of chromosomes suggested that the univalents had split, and attempts of cells to eliminate chromatin were apparent. JEFFREY and HICKS (**31, 32**) investigated meiosis in *Drosophila melanogaster* in an at-

tempt to gain some light on the origin of its "mutating" power. Both divisions showed the remarkable condition of the complete absence of a metaphase plate, although there were some paired as well as single chromosomes distributed widely on the spindle. The chromosome number was higher than expected; there was much lagging; and a large amount of chromatin was ejected into the cytoplasm. They concluded that these aberrancies were characteristically hybrid.

APOGAMY.—ROSENBERG'S studies (**49, 50**) on the highly polymorphic and apogamous genus *Hieracium* have thrown much light on hybrid cytology and the allied problems of apogamy, polymorphism, and sterility. OSTENFELD (**45**) worked on the genetical side of the genus, and furnished pedigreed hybrid material for investigation. ROSENBERG reported that the pollen mother cells in different species and hybrids acted differently: many followed the "*Rosa* type" of reduction and formed pollen with varying chromosome numbers; in others there was no pairing of the chromosomes, so that the interkinesis nuclei received very different numbers of chromosomes. A variation of this consisted in the failure of the chromosomes to pass to the poles at heterotypic division, so that the nuclear membrane was reestablished about the univalents and a "regression nucleus" formed, and dyads with the somatic number of chromosomes resulted from the homeotypic division. Most of the apogamous *Archieracia* species were found to be triploid, and followed the course last described in the division of the pollen mother cells and the embryo sac mother cells.

WINGE (**65**) and ERNST (**14**) reported that apogamy was a result of hybridization. Later ERNST (**15**) reviewed the situation, and pointed out that apogamy is linked with high chromosome number, polymorphism, and poor and impotent pollen. It is rather generally conceded now that hybridization and apogamy are at least related factors. SHARP (**55**), in discussing ERNST'S theory, expressed the opinion that "little doubt can be entertained regarding the essential correctness of the theory."

AMITOSIS.—ROSENBERG (**50**) was of the opinion that many of the descriptions of amitosis in reduction divisions which appear in the literature should be ascribed to the formation of "regression nuclei" at interkinesis, resulting from failure of the chromosomes to pass to

the poles, as he described in certain species of *Hieracium*. YASUI (67) and LJUNGDAHL (37) recognized this origin of such figures in the microspore mother cells of poppy hybrids, as BORGENSTAM also did in *Syringa chinensis* (5). Other cases of its appearance in microspore mother cells which may confidently be referred to the same source, especially as dyads commonly resulted from such cells, are the findings of SHIBATA and MIYAKE (56) in *Houttuynia cordata*, OSAWA in *Taraxacum albidum* (44), and TOKUGAWA and KUWADA in varieties of garden canna (63).

Reports of division at all suggestive of the amitotic methods described in *Selaginella mandaiana* have appeared rarely in the literature. CANNON (11) described some "amitoses" in hybrid cotton plants, which in their most degenerate phase suggest, if not the extreme condition in *S. mandaiana*, at least the somewhat similar one in *S. apoda*. WODSEDALEK (66) showed in his fig. 47 a degenerating primary spermatocyte in the mule, which appears like the more aberrant type of amitotic division in *S. mandaiana*, although its history is different. In this cell many of the chromosomes have fused into large spherical bodies which remain scattered in the cytoplasm. It should be emphasized that in these cells of the cotton plant and the mule death is imminent, and this is also the case in the tapetum of a *Ribes* hybrid described by TISCHLER (61), where the nuclei lost almost all their dark-staining substance which lay as elongated bodies in the cytoplasm. Oligopyrene and apyrene sperms, which suffer loss and degeneration of chromatin, are not considered to be functional. WARREN (64) examined the spermatogenesis of certain spiders and found surprising conditions. In the primary spermatocytes the spireme broke down into granules, which either divided into small clusters (each of which became surrounded by cytoplasm so that multispermatid formation resulted), or the chromatin granules condensed and formed a number of large masses of inconstant size and number, which resembled chromosomes (each of which became a center for spermatid formation). WARREN thought that the sperms formed by these processes were functional, as they were the exclusive method of spermatid formation in some species.

CHROMATIN IN CYTOPLASM.—Interesting comments upon the occurrence of chromatin in the cytoplasm are made by TISCHLER (62) in the banana, OSAWA (44) in *Taraxacum*, SEARS (54) in *Taraxacum*, LONG-

LEY in *Rubus* (38) and *Crataegus* (39), TOKUGAWA and KUWADA (63) in canna, and FISK in *Zea mays* (17). In many cases where no mention of them is made in the text, the figures show dark-staining bodies in the cytoplasm. JUEL (33), studying meiosis in the hybrid *Syringa rothamagensis* (*S. chinensis*), found copious chromatin in the cytoplasm at the periphery of the cell, which first appeared at diakinesis and later increased in amount. At the end of the second division these chromatin bodies became rounder, lay strewn through the cytoplasm, and appeared to form small nuclei. He suggested that the chromatin may represent the extruded chromosomes from one parent gamete, whereas those of the other divide on the spindle.

Chromatin in the cytoplasm at the divisions of the microspore mother cells in such large amounts as to be a source of confusion has never been reported, so far as the writer knows, in any plants which are not hybrids, or whose purity of inheritance cannot reasonably be suspected. This may be due to a fundamental disturbance in the metabolism of the cells, or incompatability of chromatic substances; its origin is doubtless in a mixed inheritance.

POLYMORPHISM.—That polymorphism has resulted within genera whose species hybridize freely has been shown in many groups: *Salix*, *Betula*, *Quercus*, *Rubus*, *Crataegus*, *Rosa*, *Viola*, *Aster*, *Solidago*, *Taraxacum*, *Hieracium*, many of the ferns, grasses, sedges, orchids, etc. Moreover, within some "species" great variability is found, so that they come to be regarded as collective species composed of different races, as *Marsilia drummondii* and *Taraxacum officinale*. Such cases of microspecies are commonly associated with apogamous embryo formation. TÄCKHOLM (60) thought that the apogamous rose forms are F_1 hybrids from the Pliocene era, which have continued to the present day through asexual reproduction. HOLMGREN (30) pointed out that species which are facultatively sexual, like some species of *Hieracium*, can continue to cross and produce new forms which may become fixed by apogamous reproduction.

INFLUENCE OF ENVIRONMENT.—Various investigators have experimented with the influence of external agents on cell division. SAKAMURA (52) treated anthers of *Vicia faba* with chloral solution and obtained abnormalities in division which were very similar to

those found in hybrids, mutants, and apogamous plants. By subjecting shoots to low temperature BORGENSTAM (5) produced meiotic irregularities in the hybrid *Syringa rothamagensis* (which otherwise was found normal, although JUEL and TISCHLER had found typical hybrid abnormalities in it), and MICHAELIS (42) increased the amount of abnormalities in *Epilogium angustifolium*, whereas STRASBURGER (58) found that diploid eggs developed in great numbers in *Marsilia drummondii* only at high temperatures. Adverse environmental conditions increased the percentage of formation of tetraploid gametes in KARPECHENKO's intergeneric hybrids, *Raphanus sativus*×*Brassica oleracea* (34). Some of these experiments indicate that hybrids are particularly susceptible to adverse conditions.

In considering the influence which the artificial greenhouse environment may have had on spore formation in the exotic species of *Selaginella* examined, it is significant that all the conditions and anomalies found parallels among native species; even the extreme mode of amitotic division frequent in *S. mandaiana* was observed in *S. apoda*. Hence if the greenhouse habitat exerted any influence it was of degree and not of kind.

Conclusions

By virtue of its great antiquity, *Selaginella* may reasonably be supposed to have had all the time and opportunity necessary for the development of great numbers of species by the evolutionary processes. That it has also increased and diversified its members through hybridization seems a necessary conclusion, in view of certain characteristics of many of the species which have been gradually coming to light. Adaptations for vegetative propagation, vegetative anomalies, luxuriance, polymorphism, apogamy, dyad spores, absence of microspores, sterile spores, irregular meiosis, polycary, pathological conditions in the tapetum and dehiscing layers, wholesale extrusion of chromatin in spore mother cells, much chromatin in the cytoplasm of the dividing spore mother cells, failure of mother cells to divide and lack of concerted and consistent action among them: all these conditions have been found in known hybrids, and various combinations are found in many species of *Selaginella*. That all these features are exclusively those of hybrids is not contended, but

when they are concurrently present the evidence is as decisive as may be.

Some of the apogamous species of *Selaginella* must be very ancient hybrids, as they include in their numbers *S. selaginoides*, the species which consensus of opinion has given the most primitive position in the genus, and another nearly related and comparatively simple form, *S. rupestris*. The first of these has the widest distribution of any species of *Selaginella*, and forms of the polymorphic *S. rupestris* are scattered liberally over the western hemisphere and also appear in the eastern.

Natural hybridization among plants as a source of species formation is proving to be of general occurrence. To the long list of groups in which it has been found to be widespread, the Selaginellaceae must apparently now be added.

Summary

1. Some aberrancies of habit were observed, such as bifurcated cones and reversion of the cone at the apex to a vegetative condition.

2. The method of spindle formation in both megaspore and microspore mother cells is very different from the usual one in plants. The spindle fibers appear in the cytoplasm while the nucleus, in synapsis, is at the periphery of the cell. Later the nucleus returns to the center of the cell and is enveloped by the broad spindle.

3. The mother cells are lacking in concerted action and many fail to divide.

4. The study of meiosis is difficult for three reasons: the scarcity of divisions; the extreme smallness of the mother cells; and the quantity of chromatin-like bodies frequently found in the cytoplasm, which in many instances cannot be distinguished from chromosomes.

5. In *S. amoena* the heterotypic division is irregular.

6. In *S. apoda* the heterotypic division is often slightly irregular.

7. Two aberrant types of division are predominant in *S. mandaiana:* (1) the contents of the nucleus pass out into the cytoplasm, the cell divides somewhat quantitatively, and new nuclei are organized; (2) the nucleus elongates and the chromatin is divided without chromosome formation.

8. *S. rupestris*, which is apogamous, is almost exclusively mega-

sporangiate, has a comparatively high chromosome number, and forms its microspores in dyads like many apogamous angiosperms.

9. There is a large percentage of sterility in the spores of the species of *Selaginella* examined. Collapsed microspores, microspores devoid of contents but with perfectly sculptured exine coats, megaspores and microspores with discolored exine coats, small granules of exine material scattered through sporangia, and collapsed sporangia are common features.

10. No advanced stages of development of male gametophytes were seen except in occasional spores of *S. apoda*.

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STUDIES ON THE MORPHOLOGY OF THE ONAGRACEAE

II. EMBRYONAL MANIFESTATIONS OF FASCIATION IN CLARKIA ELEGANS

DONALD A. JOHANSEN[1]

(WITH FIFTY-TWO FIGURES)

Introduction

The literature of botany abounds in descriptions of teratological seedlings, cotyledonary aberrations, and similar phenomena (13). It seems, however, that practically all observations have been made on nearly mature plants, *after* fasciations as such became visible. From the observation of germinating seeds of various Onagraceae during the past few years, it has become increasingly apparent that many of these phenomena had their origin during the early development and organization of the embryo. This paper is intended more as a contribution to the study of the origin of fasciation phenomena than as an attempt to explain these anomalies.

In *Clarkia elegans* Dougl. fasciation is a phenomenon commonly observed in pedigree cultures conducted over a period of many years, apparently being carried over from one generation to the next. It is still uncertain with what this heritable tendency is linked or otherwise correlated genetically. According to experiment garden records, compiled over a period of several years, those cultures which possess a high proportion of fasciation show in their seedlings a correspondingly high percentage of cotyledonary abnormalities. Since the cotyledons of many onagrads are decidedly characteristic of the species, and of certain genera as well, any variations from the normal are observable as soon as the modified leaves bearing the cotyledons become fully expanded. In itself, however, this is hardly an infallible sign of approaching fasciation, for the first definite external indications of this teratological condition become noticeable in irregular phyllotaxy preceding the appearance of the inflorescence. It is therefore extremely likely that genes other than those primarily responsi-

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ble for fasciation are the basic cause of simple cotyledonary anomalies.

In 1925 I happened to collect, for embryological study, a number of ovaries from a strongly fasciated plant of *Clarkia elegans*. When sectioned and examined two years later, considerable irregularity in the succession of divisions in the young embryo was noted. This strengthened the supposition just expressed, and suggested that the genesis of fasciation, whether it is to become manifest shortly after germination or during the development of the inflorescence, was to be found in the embryo, even during the formative stages preceding growth of the cotyledons. Further, since the time of SACHS, it has been assumed by plant physiologists that fasciations may readily be produced artificially by mechanical injury, provided such stimulus be applied immediately after germination and that it affects only the initial meristem. If such be the case, would it not be probable that in the ordinary course of events something might occur in the zygote which would so disturb the rhythm of the regular sequence of divisions that fasciation and other abnormalities would become apparent after germination? Preliminary observations on the 1925 material showed that this could readily happen, and consequently it was thought worth while to undertake a special study of the early embryogeny of plants from certain pedigrees long known to possess a decided tendency toward both teratological cotyledonary phenomena and fasciation.

This study has been made on material collected principally during 1928 from the cultures of Dr. L. L. BURLINGAME at Stanford University, who very generously placed his plants at my disposal.

Material and methods

An entire series of ovaries, representing all stages in the development of the embryo, were collected several times over from specially selected individuals in many pedigrees. The most satisfactory killing fluid was that of Petrunkewitsch; the ovaries were cut into convenient lengths, the air removed by means of a hand pump, and after being imbedded were soaked in water for several days and sectioned longitudinally at 10 μ.

Ovule and mature megagametophyte

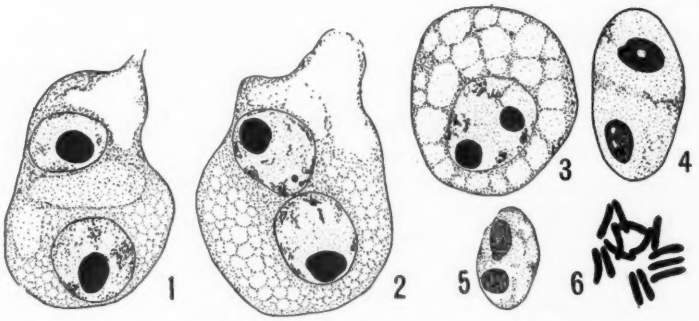
Ovaries from fasciated branches are always shorter and broader than normal ovaries, and the ovules are somewhat smaller. There is no reduction in the number of ovules, consequently the seeds acquire a sharper angularity from mutual pressure. Perhaps on account of the greater supply of water present in fasciated branches, the hypostase (**8**) is much reduced; an epistase has not been observed, except in a single anomalous instance. No irregularities were noted in the development and organization of the megagametophyte, which, at maturity, is of the regular tetranucleate type characteristic of the family. It is somewhat smaller, however, and occupies less space in the embryo sac cavity. Moreover it is apparently a very fragile and unstable structure physiologically, as it collapses and degenerates with comparative ease and frequence.

Fertilization

For some reason not completely understood, fertilization proceeds unusually slowly in the ovules of fasciated plants of several other onagrads besides *C. elegans*. It is consequently easy to follow the various stages in the entrance of the microgametophyte and the union of the two male nuclei with the egg nucleus and the polar nucleus respectively (figs. 1–3).

In most published figures of onagrad megagametophytes the egg cell appears as if it were a perfectly rounded sphere; at any rate, one scarcely fails to acquire the impression that the egg cell is completely inclosed by a wall or membrane. Such is hardly the fact; in each of over fifty species whose egg cells were examined the cell was found to be pyriform, with the narrowed neck pointed toward and between or over the synergids. The neck is either open or superficially closed by an extremely thin membrane. The male nucleus invariably enters through this opening or through the easily ruptured membrane: no other mode of entrance was observed. At first the male nucleus is surrounded by a thick mass of cytoplasm, part of it coming from the microgametophyte and the remainder from the synergid or synergids demolished during the entrance of the former, but this alien cytoplasm becomes almost wholly divested from the male nucleus in the

upper part of the egg cell, where it mingles with remnants of the egg cytoplasm displaced from below by the entering male nucleus (fig. 1). This commingled cytoplasm is doubtless shut away from the lower part of the cell after the first zygotic mitosis, but it is within the realm of possibility that some of it is brought into the lower portion of the egg cell, and that it may have an effect upon the future behavior of the zygote.



FIGS. 1–6.—Figs. 1–3, syngamy: fig. 1, entrance of male nucleus, bringing plasm from microgametophyte and demolished synergids; fig. 2, later stage; vacuole in upper part of egg (still visible in previous figure) has disappeared; fig. 3, fusion of nucleoplasms complete; nucleoli still distinct; fig. 4, union of secondary male nucleus with polar nucleus, illustrating normal type; dissolution of nuclear walls nearly complete; fig. 5, type in which male nucleus resembles spermatozoid, showing male nucleus fusing first with smaller polar nucleolus (visible as "bud"); fig. 6, chromosomes of first zygotic mitosis; ×1250.

The male nucleus is but slightly smaller than that of the egg; in fact, the two often cannot be distinguished except by the relative position in the egg cell, the nucleus of the latter being generally the lower. The two sexual nuclei are both in the reticulate condition during the entire course of the fusion process; no metabolic changes were observed or have been recorded by others (fig. 2). The union of the nuclei (in these fasciated plants only) may be described as a leisurely process; the male nucleus slowly approaches that of the egg and syngamy proceeds deliberately. After the nuclei become well pressed together, the walls separating them are slowly absorbed into the nucleoplasm of the fusion nucleus (fig. 3). The nucleoli remain distinct for some time, finally fusing. Most writers agree that the

nucleoli fuse, but RENNER (15) contends that in *Oenothera lamarckiana* one of the two "dann zugrunde gehen könnte."

The fact should be emphasized that syngamy in *C. elegans* consists of two distinct phases: fusion of the nucleoplasms first, followed by fusion of the chromatin-containing nucleoli. Furthermore, infinitesimal particles of foreign plasm are rather certain to be adherent to that part of the wall of the male nucleus absorbed into the fusion nucleus. These facts are significant.

The secondary male nucleus is ordinarily similar to the primary male nucleus, and fuses with the polar nucleus while both are in the reticulate condition. The polar nucleus is sister to that inclosed within the egg and contains the haploid number of chromosomes. In nine cases out of ten the polar nucleus is binucleolate, no matter whether the plant from which the ovaries were secured was teratological or not. The size of the smaller of the two nucleoli ranges from 5 to not more than 20 per cent of the bulk of the larger, and it is usually oriented in the upper portion of the nucleus toward the egg cell. It generally fuses with the larger previous to the union of the nucleus with the secondary male nucleus. As in the egg cell, the nuclei first fuse, followed by fusion of the nucleoli, but both processes are consummated with far greater rapidity (fig. 4). Immediately there is initiated a series of simultaneous rapid divisions of the primary endosperm nucleus so created, the successive generations of nuclei gradually diminishing in size until they become mere fragments of chromatin and division is no longer possible. The "endosperm" finally disintegrates completely and no trace of it can be found in mature seeds.

In some instances the secondary male nucleus assumes an entirely different character, possessing the same size, shape, and staining reactions as the nucleolus of a normal nucleus. It was not possible, in many hundreds of ovules, to follow the progress of the nucleus from its entrance into the embryo sac to the vicinity of the polar nucleus, hence exactly what occurs to cause its changed character is not known. In a number of undischarged microgametophytes only normal male nuclei were observed. In any event, the behavior of this nucleus after reaching the polar nucleus has been traced closely: it penetrates the nucleus, fuses first with the smaller nucleolus if the

latter has not already united with the larger, and then with the larger, after which normal division occurs (fig. 5). The number of nuclear divisions subsequently taking place is approximately half the normal, owing to the obvious fact that the bulk of the primary endosperm nucleus is less than half that of a normal one. It had been recognized for some time that in many embryo sacs there was half the quantity of "endosperm" nuclei and cytoplasm as in others: the unusual behavior just described furnishes a satisfactory explanation of this phenomenon.

This is admittedly a most peculiar mode of behavior, and one which apparently has not been reported for any other onagrad. Its significance is probably indicative of a further stage in the gradual reduction in importance of the "endosperm" in that order of the Dicotyledoneae (Myrtales) under which the Onagraceae are placed, and further brings out the relation of the male nucleus to this reduction. Another significant fact is that embryo sacs with well developed normal embryos but lacking every vestige of "endosperm" are frequently encountered in material from both normal and fasciated plants.

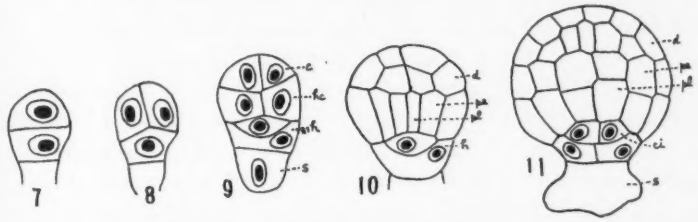
The time elapsing between pollination and fertilization averages 36 hours in normal plants and 44 hours in fasciated plants. The reason for this 8-hour difference requires further investigation.

Normal embryonal histogeny

It was naturally necessary to investigate first the development of the normal embryo in the species under discussion, in order to ascertain if the sequence of divisions was regular, and to provide a basis for comparison. For convenience, the detailed histogeny of the embryo may be given, as worked out by Souèges (**16**) for *Oenothera biennis*, and confirmed by Johansen (**7**) for *Godetia amoena*. The development of the embryo and its tissues from the zygote onward is a remarkably uniform process among all species of the family so far investigated.

Following the primary transverse division in the zygote there occurs a longitudinal segmentation of the apical cell, then a transverse partitioning of the basal cell. The two juxtaposed upper cells are destined to give rise to the cotyledons, stem apex and hypocotyl, the

intermediary cell becomes the hypophysis, while the lowest forms the suspensor (fig. 7). The two upper cells divide once longitudinally (at right angles to the first wall) to form the quadrant stage (fig. 8). A transverse segmentation of each quadrant initiates the octant stage (fig. 9). The four superior cells of the octant correspond to the cotyledonary part of the embryo (*c*); the four inferior cells represent the hypocotyledonary portion (*hc*). During the differentiation of the octants the lowest cell (*s*) divides into two which generally do not divide again, although sometimes the lowest increases so greatly in size that it appears hypertrophied. These two cells constitute the suspensor (fig. 39). In several species, although somewhat rarely in



FIGS. 7–11.—Histogenesis in onagrad embryo as illustrated by *Godetia amoena*; ×450.

C. elegans, the suspensor becomes separated from the body of the embryo and withers (fig. 44). There is considerable difference among the species regarding the time at which the hypophysis cell (*h*) divides. Although this statement should not be regarded in any sense as dogmatic, the hypophysis usually divides at the same time that the octants are being organized. The partition is peculiar in that it is concave from above and separates the original cell into two unequal cells; the latter, by means of two vertical divisions, give rise in turn to two groups of four superposed cells (fig. 11 *ci*). In several cases the hypophysis cell had at the beginning two rectangular partitions separating four "hypophysis quadrants" which, by horizontal segmentation, contribute in the same way to the two groups of four superposed cells. The upper group of four cells represents the initials of the cortex (*ci*); the lower group, by vertical, then tangential partitions, gives rise to the two primary layers of the root cap. This last

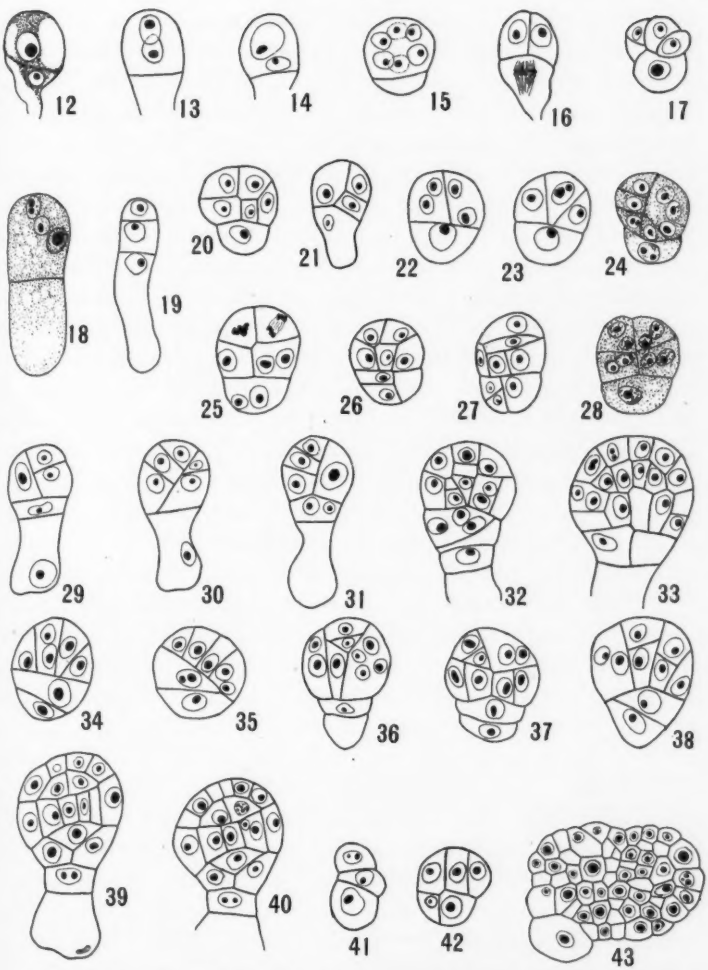
tissue becomes extended to the right and left by tangential segmentations of the dermatogen (*d*) of the hypocotyl axis, and increases in thickness by centripetal divisions of the innermost layer, becoming the calyptrogen layer. The outer layer of cells covering the embryo at this stage constitutes the dermatogen, the next inner group forms the periblem (*pe*), while the innermost gives rise to the plerome (*pl*). Further development proceeds in the usual method characteristic of the Dicotyledoneae. SOUÈGES truly concludes that "it is the precocious differentiation in the hypophysis cell which sharply differentiates the embryo of *Oenothera*."

ZYGOTE AND YOUNG EMBRYO

Owing to the immense number of buds on fasciated plants and to the slowness of fertilization, not more than 80 per cent of the ovules in any particular ovary are fertilized. Nevertheless, zygotes are always found in abundance; the comparatively low proportion of unfertilized ovules in fasciated material to the high percentage of degenerated megagametophytes in normal ovules makes zygotes easier to find in the former. The first postsyngamic mitosis was twice observed (fig. 6); fourteen chromosomes were easily counted in each (in regard to the number of chromosomes in *C. elegans*, see JOHANSEN 9). In a few cases the zygote nucleus had a distinctly amoeboid appearance, consisting of a large, irregularly outlined central portion from which a few broad protuberances extended to the periphery of the nucleus. On the whole, the zygotes and the first mitosis therein show no appreciable difference in comparison with strictly normal zygotes; it is immediately after the primary mitosis that irregularities first become apparent. A certain proportion of the zygotes (ranging from one or two in each ovary to 75 per cent of the total number, depending upon the pedigree) possess two nuclei (figs. 3, 4).

Binucleate (or rarely trinucleate) cells are one of the principal characteristics of embryos from fasciated plants of *C. elegans* (figs. 22, 25, 32, etc.). How well this holds for other onagrads cannot be stated at present, as there has not been the opportunity of collecting fasciated material in sufficient quantity from other species.

Although so far as could be ascertained the first wall in the zygote is transverse, as is invariably the case in normal fertilized egg cells,



FIGS. 12–43.—Fig. 12, failure of nucleus to divide following primary zygotic mitosis; plasmolysis and beginning of degeneration (common occurrence); figs. 13, 14, nuclei retained in upper cell following primary zygotic mitosis; fig. 15, free-nucleate unicellular embryo; fig. 16, apparently normal embryo, with mitosis (14 [= $2n$] chromosomes) in suspensor cell; fig. 17, irregular young embryo; figs. 18, 19, filamentous embryos, first being free-nucleate (note difference in size, although both embryos came from same ovarian locule); figs. 20–38, various stages in growth of anomalous embryos (cf. suspensors of figs. 29–31 with those of figs. 34–38); figs. 39, 40, normal embryos from strongly fasciated plants; figs. 41–43, stages in development of unilateral embryo; ×450.

the wall is often formed in such a manner that both nuclei are retained in the cell destined to form the embryo proper, thus leaving what should be the initial cell of the suspensor without a nucleus, such a zygote constituting the binucleate form previously mentioned. Between the two a wall may later be formed in the cytoplasm; the upper cell is the embryo initial, while the lower in later behavior simulates the appearance of a suspensor, but is otherwise ontogenetically indistinguishable from the embryo proper. It is quite possible that the irregular suspensors of embryos such as that shown in fig. 36 arose from a cell of the peculiar origin so described. The second division is vertical as usual, but is hardly ever exactly perpendicular to the primary wall; instead it is more or less oblique and usually considerably to one side of the median line (fig. 22). Often a second vertical partition, close to the first at the base but far apart at the periphery, is formed shortly after the first (fig. 23), and sometimes three or more walls may be erected in the upper cell (figs. 34, 35). Generally speaking there is a conspicuous absence of order, sequence, and direction in the formation of all cell walls except the first transverse partition. It is rare that any semblance to normal order is attained (fig. 33); at any rate there are in such cases appearances which stamp the young embryo as teratological, the most decisive of which is the presence of binucleate cells. Strictly normal embryos are always to be observed in ovaries from fasciated plants (figs. 39, 40).

Unilaterality and spurious monocotyledony

Seedlings with only one normal cotyledon, and possessing or lacking the rudiments of the second, are frequently found in large batches of seedlings, and it was consequently not surprising to find abundant evidence of the origin of this condition in developing embryos. These embryos are especially interesting in view of their possible relation to the controversy as to whether monocotyledons were derived from dicotyledons, or vice versa.

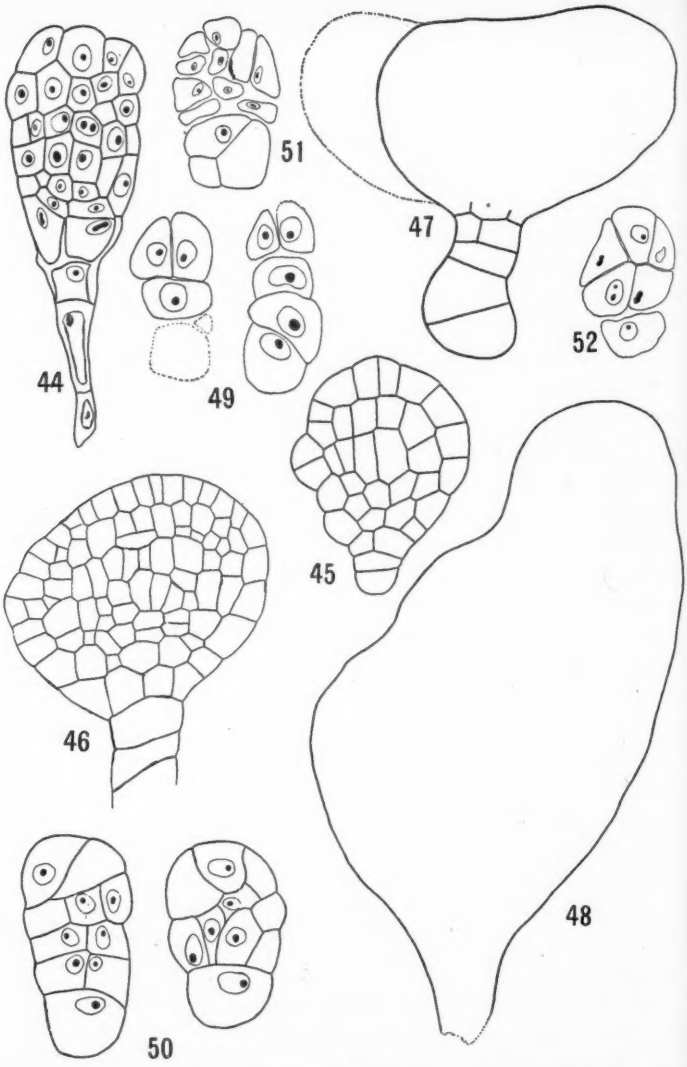
The earliest stages of the condition here described as unilaterality (or, to employ another term of similar but more significant meaning, spurious monocotyledony) are easy to detect (figs. 41–43); there seem to be two or three different types. In some cases, before the second vertical division takes place, one of the side cells is divided

more or less equally by a transverse wall (figs. 29, 31, 36, 38), and a second division occurs shortly after in the upper daughter cell. In the other cell of the 2-celled stage the normal vertical wall is sometimes formed, but a horizontal wall similar to the first in the neighboring cell is usually erected. In other types unilaterality is produced when there is an excessive number of longitudinal partitions in the apical cell and an unequal division (premature formation of the hypophysis cell?) in the basal cell (figs. 23, 34, 35). Still other cases (figs. 17, 41–43) are irregular from the beginning.

When there is order in the succession of divisions of a unilateral embryo, the result is the formation of an embryo normal in all respects save that it possesses only one normal cotyledon and the rudiments of the second (figs. 45, 46, 48). On the other hand, when there is absence of order in the divisions, an indefinite multicellular body is formed (fig. 43), which finally disintegrates. Sometimes such a structure may be found in a healthy condition in maturing seeds, but it is incapable of germination.

Fig. 43 further represents a type of embryo-like structure rather commonly encountered. Except for the large "suspensor" cell, these structures are not very different from similar embryos found in non-fasciated plants, the principal differences being that those from fasciated plants have in the aggregate a larger number of cells but lack a corresponding increase in size of the entire structure. From the large number of disorganized and disintegrating structures of this type observed, it is presumed that a lethal factor may be linked with the factor causing the formation of these embryos, thus insuring the eventual death of the monstrosities. The conspicuously large "suspensor" cell sometimes persists in an apparently healthy condition long after the remainder of the structure has completely degenerated.

The behavior of the "suspensor" deserves special notice (cf. figs. 29–31 and figs. 34–38). The only possible explanation is that, in the latter group, the primary suspensor cell failed to undergo division. In figs. 35 and 38 the diagonal wall is patently that which should have formed the concave wall in the hypophysis cell, but which failed to become concave so as to connect with the primary transverse wall at both sides.



FIGS. 44–52.—Fig. 44, columnar type of developing embryo: genesis of stem fasciation, with evidence of later split stem; fig. 45, beginning of growth (tricotyledony?) on one side of otherwise normal embryo; fig. 46, later stage of similar type; fig. 47, outline of tricotyledonous embryo; fig. 48, outline of monocotyledonous embryo (early stage in development of normal cotyledon purposely drawn to accentuate position of stem apex and aborted cotyledon); fig. 49, apogamous embryo, two successive sections; degeneration just commencing; fig. 50, same, healthy and showing no signs of disorganizing; figs. 51, 52, disorganizing apogamous embryos; ×450.

Tricotyledony

This phenomenon is frequently encountered in seedlings, but the difficulties of demonstrating its appearance in the embryo with a tolerable degree of certainty are almost insurmountable. One unquestionably tricotyledonous embryo is shown in fig. 47.

Diploid apogamous embryos

The definite presence of these structures in one pedigree has been established. The diploidy was ascertained by counting at least twelve chromosomes in each of two mitotic figures. In structure the embryos are irregular, tending to be filamentous, and usually begin to disintegrate upon reaching about the 16-celled stage (figs. 49–52). Their origin may be attributed to the failure of the megasporocyte to undergo meiosis.

Parthenogenesis is unknown. Adventitious embryos have been observed only once, when two were found side by side on the under side (toward the embryo sac) of the only epistase found in an ovule from a fasciated plant.

Discussion

So far as can be ascertained, there is no paper in the literature on the embryology of the Dicotyledoneae which discusses the genesis of fasciation, of known cotyledonary irregularities, or of other postgerminal teratological phenomena in the embryo. The reason for this apparent deficiency is found in the fact that it is rather well agreed that these irregularities as such are not identifiable until some time beyond germination; hence it seems to have been presumed that they could not be observed in earlier stages of development.

Several opinions to account for the causes of fasciation are current, but the validity of none has been satisfactorily or conclusively demonstrated experimentally. It may be interesting to examine a few of the conjectural theories in connection with the facts here presented.

De Vries (20) pointed out that the first leaves appearing after the cotyledons make it possible to recognize a mutant form in *Oenothera*. This fact is also mentioned by Gates (5). Evidently the factors responsible for the creation of the new mutant begin to exercise their influence early in the life of the embryo; hence it would be interesting to study the comparative embryogeny of mutative forms of *Oeno-*

thera lamarckiana. One fact contributory to the early recognition of mutation forms is that only hypogeal seedlings exhibit fasciation. WHITE (**22**) thinks that (in *Nicotiana tabacum*) "the actual place in ontogeny at which the change from the normal to the abnormal took place must have been shortly after fertilization. If it had occurred later in ontogeny, the fasciated character would have appeared first as a bud-sport." As noted by WHITE, the idea that teratological variations originate more frequently under artificial conditions than in nature is not supported by the facts. He states that "unless the situation were considered carefully, one might conclude prematurely that in these plants the anomalous character is reproduced through seed," but does not know that this has been factually demonstrated. It is to be hoped that the observations recorded here may shed some light on this question, although they were not primarily directed toward this end.

In a few plants belonging to heterozygous pedigrees of *C. elegans*, fasciation and total inhibition of the inflorescence may occur simultaneously; or if buds are formed at all, they soon drop off. WHITE (**22**) cites GODRON to the same effect (in *Onagra biennis*). During several successive late summers this phenomenon has been common in colonies of *Epilobium angustifolium* on Fickle's Hill, east of Arcata, California. Many of these colonies are undoubtedly natural clons, and the total repression of the inflorescence in a high percentage of the ramets in each clon, whether or not fasciation is also present, points directly to the probability that the origin of the phenomenon, as well as of clonal colonies themselves, lies in the suppression in the ortet[2] of the faculty for the formation of sporogenous cells. The latter phenomenon is actively proceeding in many species of *Godetia*, particularly in *G. dudleyana* and *G. grandiflora*, but in these plants it leads merely to the eventual extinction of the species since they have not developed a faculty for vegetative reproduction.

No positive cytological evidence could be adduced in support of ATKINSON'S (**1**) statement:

> The material representing the group of characters not entering into any one working combination, may then be left behind in the first suspensor cell of the embryo, or cast out into the cytoplasm, or may become subordinated. The material in the first suspensor cell, as is well known, plays no part in the formative

[2] For an explanation of the terms clon, ramet, and ortet, see STOUT, A.B., Jour. N. Y. Bot. Gard. **30**:25–37. 1929.

processes of the new individual, since this cell is sidetracked by the basal wall formed during the first division of the zygote.

Nevertheless, it is wholly within the bounds of possibility that any one of the three suppositions expressed by ATKINSON may yet be demonstrated by cytological evidence.

Some attention was devoted to SWINGLE's zygotaxic theory (**17**), but it was soon found impossible to apply this experimentally, as the very small chromosomes of *C. elegans* are morphologically so much alike that any particular pair of homologous chromosomes cannot always be distinguished from the others (fig. 6). Furthermore, as ATKINSON states: "There is . . . [no] . . . cytological evidence that the positional arrangement of the chromosomes in the cells of hybrids bears any relation to their morphological characters."

One subject closely related to that under discussion is the behavior in the embryo sac when species belonging to different subgenera of *Epilobium* are crossed. It was early discovered that whereas the cross in one direction produces normal embryos, the reciprocal cross causes the formation of an abnormal embryo or none at all. These abnormal embryos invariably degenerate, or at least are incapable of germination, after attaining the 12-celled to 20-celled stage. The cytomorphological literature on *Epilobium* crosses is too extensive to be cited here.

During this study, the possibility of predicting the appearance of teratological phenomena was demonstrated to possess a factual basis. Embryos in certain 1926 pedigrees were found to behave erratically, although the garden records indicated no irregularities of any nature. In 1927, however, the progeny from various plants of this pedigree exhibited a surprising array of aberrations, and a study of their embryology indicated that the same abnormalities would be continued during 1928. This expectation was abundantly fulfilled.

There exists considerable reason to believe that once a certain abnormality or group of concomitant abnormalities is acquired, it persists through several generations and is probably never wholly lost. There seems to be little choice in describing it as a retrogressive or an advancing evolutionary step, for it is likely to be neither: it is simply a changeable phase. As a matter of fact, there is little or nothing upon which to base a denial that so-called "abnormalities" are not in reality inseparable adjuncts to the normal existence of the

species, or, to employ a more properly descriptive term, of the linneon. Although most earlier workers considered fasciations to be in the nature of acquired characters, there is now much evidence that if fasciation is not directly a heritable character, at least a strong tendency toward the production of fasciations is carried over from one generation to the next in many species.

DE VRIES (20) summarizes the case with sufficient succinctness: Monstrosities are often considered as accidents, and rightfully so, at least as long as they are considered from the morphological point of view. Physiology of course excludes all accidentality. And in our present case it also shows that some internal hereditary quality is present, though often latent, and that the observed anomalies are to be regarded as response of this innate tendency to external conditions.

Summary

1. The genesis of various teratological phenomena is to be found during embryogeny.

2. The possible appearance of such abnormalities in progeny may be ascertained in advance.

3. They represent neither advancing nor retrogressive evolutionary steps, but in the end contribute to the changing evolutionary status of the species, for they are heritable; but fasciations caused by mechanical injury (for example, insect attacks) are not inherited.

I desire to record my great indebtedness to my Fellowship sponsors, Dr. R. A. HARPER of Columbia University, and Drs. GEORGE J. PEIRCE and L. L. BURLINGAME of Stanford University.

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A NEW SPECIES OF CUPRESSINOXYLON (GOEPPERT) GOTHAN FROM THE JURASSIC OF SOUTH DAKOTA

H. J. Lutz

(WITH THIRTEEN FIGURES)

The two specimens of silicified coniferous stems here described were collected in August, 1927, by Professor Wieland, from the *Barosaurus* horizon (Upper Como) at points two and four miles north from the town of Sturgis, South Dakota. Both were closely in place and therefore are of late Jurassic age. These fossil woods were given the writer for study in 1927. The paleobotanical laboratory at Yale University was available, and all of the section cutting, as well as the photomicrographic part of the work was done there. Ten thin sections of the type stems were cut: three transverse, three tangential, and four radial. Also, through the kindness of Professor Record, numerous wood specimens of living conifers were obtained from the Yale collection for comparative study.

Description

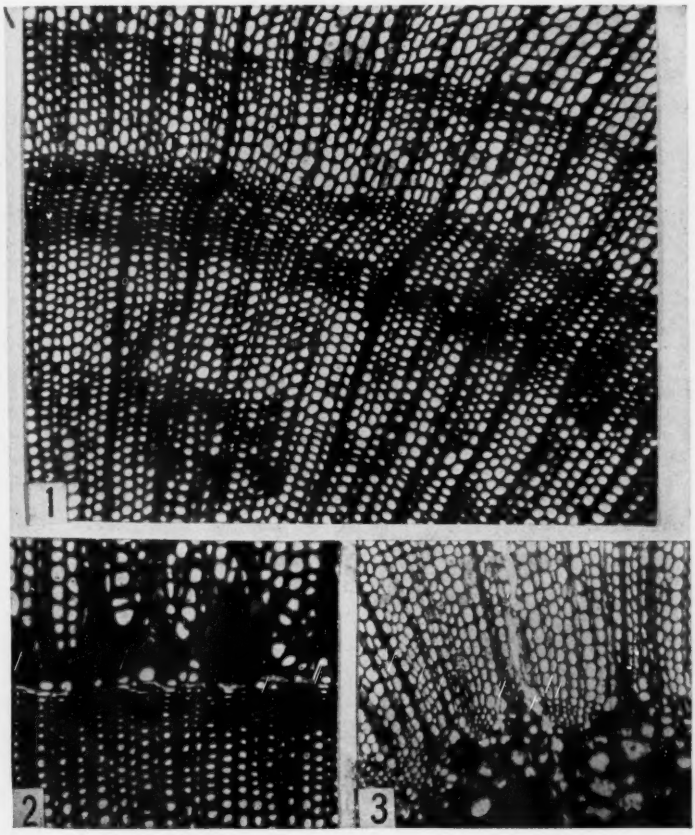
Cupressinoxylon (Goeppert) Gothan

Cupressinoxylon **jurassica** sp. nov.

Tracheids.—The preservation is exceptionally good, silicification being of the type with left-over traces of carbon. The growth rings are notable for their complexity and show a slight tendency to be sinuate; there is also considerable ring discontinuity. The wood appears to be biannulate; that is, there can be distinguished a long growth period beginning in the early spring and running into a late summer ring, with a lesser band of fall growth ending in a sharp winter ring (figs. 1, 12). Viewed in transverse section, the spring wood tracheids appear irregularly hexagonal to nearly square in shape, and show a tendency toward tangential compression. The summer wood tracheids tend to be nearly square, although usually somewhat compressed in radial diameter; rarely they are of irregu-

larly hexagonal form. The walls are often thick, causing a reduction in size of the rounded to oval lumina.

Bordered pits on the radial walls are large, strictly uniseriate, nearly round in shape (figs. 6, 8). They may be either close set or dispersed, but occupy about the entire tracheid width. The pores are usually large and round, but occasionally are lenticular. Pits



FIGS. 1–3.—*Cupressinoxylon jurassica:* fig. 1, transverse view showing mid and late summer, followed by fall growth; winter ring and spring growth above; fig. 2, parenchymatous groups of wound tissue; fig. 3, groups of primary elements adjacent to pith; ×85.

on the tangential walls occur only sparsely, and are somewhat smaller than those of the radial walls (fig. 5). They usually occupy from one-third to one-fifth of the width of the tracheid. Very faint, apparently spiral markings are distinguishable on the tangential walls of some tracheids (fig. 4). Bars of Sanio appear normal on the radial walls of the tracheids. Trabeculae are sometimes present in radial series, especially in the spring wood tracheids.

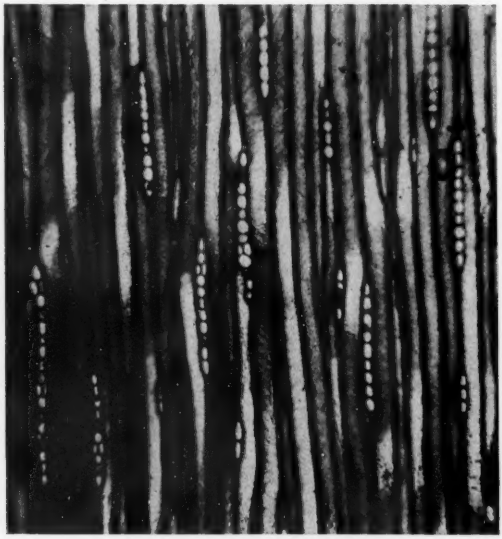
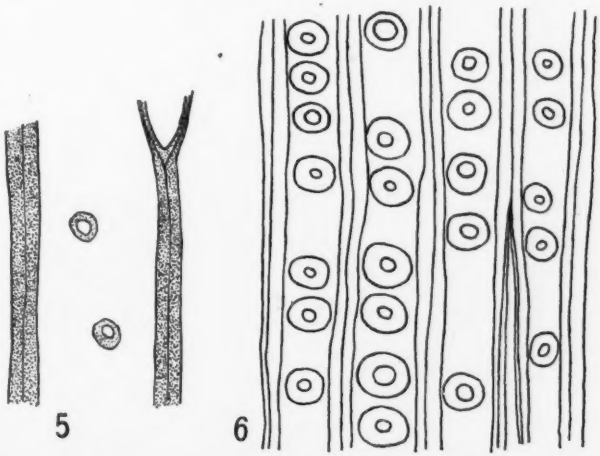


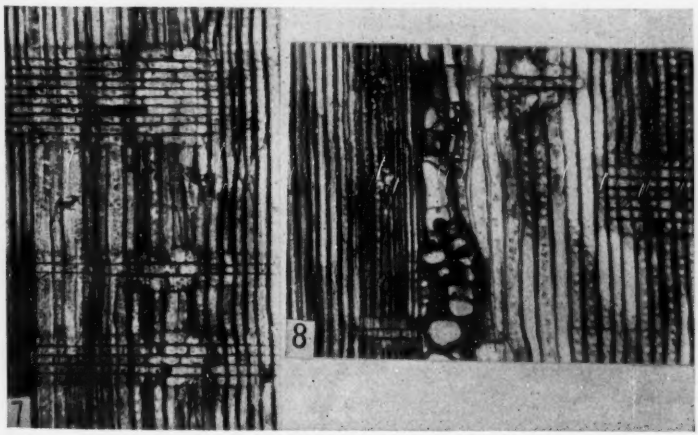
FIG. 4.—*C. jurassica:* tangential view; ×85.

WOOD PARENCHYMA.—Resin cells are numerous and usually scattered, although occasionally tending toward zonation. In transverse section they appear to be about the same size as the tracheids but are more nearly square. The parenchyma cells often contain spheroid or elongate masses of black material, no doubt originally of a resinous character (fig. 10). Terminal walls are smooth and unpitted. Small bordered pits similar to those in the walls of the ray cells occur in the lateral walls of the parenchyma cells. The pores are lenticular or almost round. No resin canals are present, but there is a well defined peripheral development of wound tissue

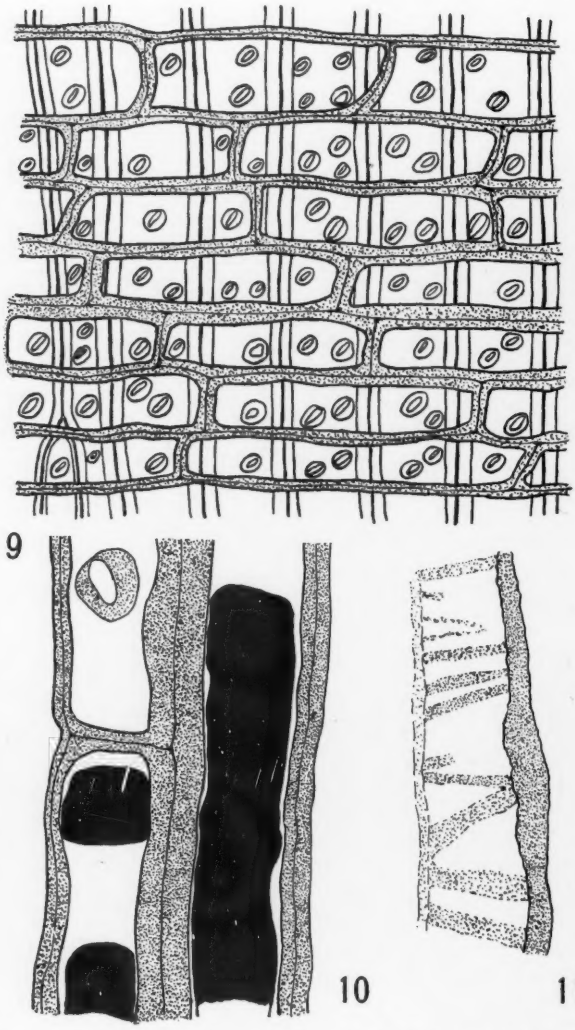
(Wundholz) in the spring wood of one growth ring (figs. 2, 8). This traumatic tissue takes the form of parenchymatous groups, and is especially developed in the region of each ray.



FIGS. 5, 6.—*C. jurassica:* fig. 5, pitting on tangential walls of tracheids; ×300; fig. 6, pitting on radial walls of tracheids; ×190.



FIGS. 7, 8.—*C. jurassica:* fig. 7, radial view; fig. 8, radial view showing wound tissue and pitting; ×60.



FIGS. 9–11.—*C. jurassica:* fig. 9, radial view showing bordered pits in ray cells; ×200; fig. 10, radial view of parenchyma cells; ×700; fig. 11, primary element with spiral thickenings on radial wall; ×700.

WOOD RAYS.—These rays are prominent, and in transverse section are four to twenty-one tracheids apart, the average distance

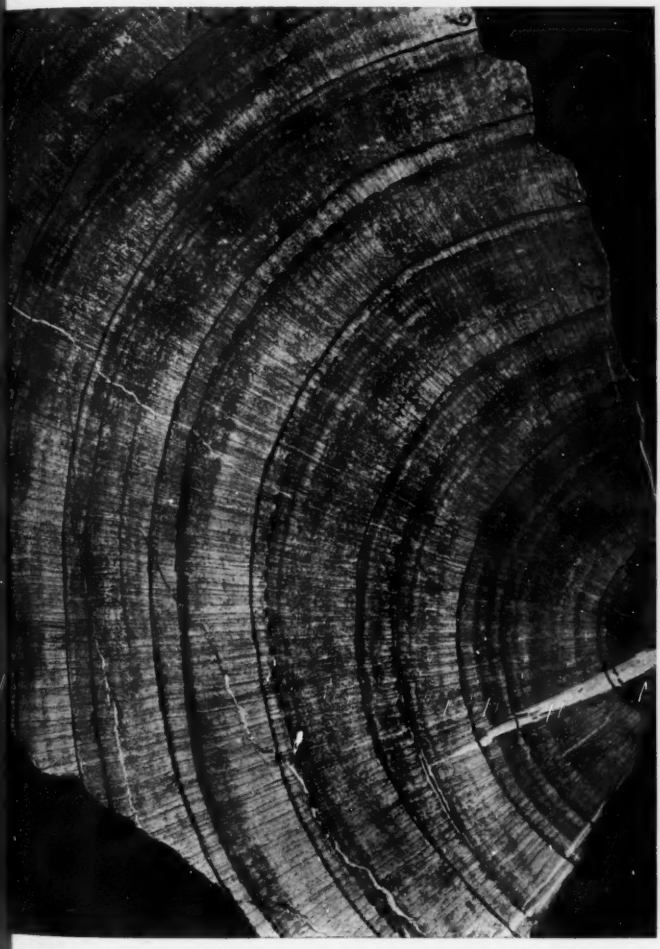


FIG. 12.—*C. jurassica:* transverse section showing combined seasonal and annual ring features. A tendency to rapid spring growth, followed by slow growth and checking during late summer dry weather, with active late fall growth and sudden winter cessation is suggested. Some of annulate shading may record the so-called "chemical growth rings" (*cf.* fig. 13). Eight years of growth are traversed by section. Late summer ring is dotted, and late fall ring is solid line; ×9.5 (photograph by G. R. WIELAND).

being eleven (figs. 1, 3). They are predominantly uniseriate, but many are biseriate in part (fig. 4). The ray cells are often slightly

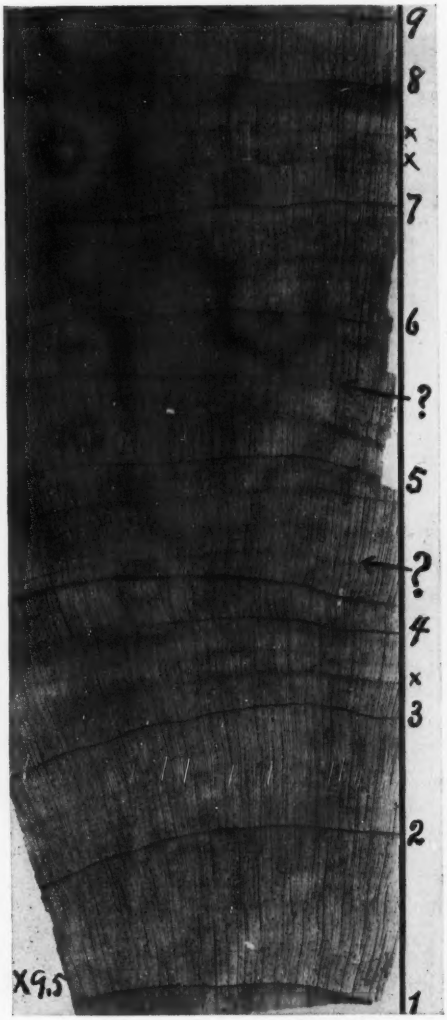


FIG. 13.—*Juniperus* sp., Cuba: transverse section showing complexity of seasonal annulation comparable to that of Como stem (fig. 12). As in that stem, both inequality and discontinuity appear. Such discontinuous and multiple rings are sometimes seen in rain forest conifers (*Araucaria*, Australia); ×9.5 (photograph by G. R. WIELAND).

contracted near their ends, and may contain masses of black material (fig. 9). Seen in tangential section these cells are broadly oval or nearly round in shape. The tangential or terminal walls are relatively thin, unthickened, and usually curved, although not uncommonly straight (figs. 7, 9). Pitting on the lateral walls is of the "cupressoid" type, that is, the pore is relatively large, the border relatively small, and at least in the spring wood the pore is usually set in an oblique position (fig. 9). In the summer wood the pores are invariably set in a vertical position, that is, parallel to the tracheid walls. There are from one to four pits per tracheid field (Kreuzungsfeld) but the usual number is two (figs. 7, 9). The pits are nearly isodiametric, although some may be oval. The pores are usually lenticular but occasionally round. In height the

rays vary from one to twenty-four cells, the average height being seven. The number of rays per square millimeter of tangential surface varies from 9.6 to 21.7; the average is 14.8. The rays are homogeneous; fusiform rays are absent.

TABLE I

MEASUREMENTS OF WOOD ELEMENTS IN CUPRESSINOXYLON JURASSICA

MATERIAL	MINIMUM (MM.)	MAXIMUM (MM.)	AVERAGE (MM.)
Growth rings			
Width of rings	0.199	3.005	1.607
Width of spring wood			1.321
Width of summer wood			0.284
Tracheids			
(spring wood)			
Length	2.639	5.976	3.669
Radial diameter			0.0315
Tangential diameter			0.0266
Thickness of walls			0.0050
(summer wood)			
Radial diameter			0.0149
Tangential diameter			0.0259
Thickness of walls			0.0080
Wood rays			
Vertical diameter of cells	0.0198	0.0252	0.0227
Tangential diameter of cells	0.0180	0.0234	0.0193
Length of cells	0.0756	0.1944	0.0936
Thickness of lateral walls			0.0036
Bordered pits			
Diameter in ray cells			0.0078
Diameter in tracheids			0.0156
Pith			
Diameter			0.2000
Diameter of cells		0.103	
Wood parenchyma			
Length of cells			0.2466

PITH.—Cells are irregularly four- to six-sided, often containing black masses, no doubt originally of a resinous nature. The walls are thickened, probably due in part to the course of petrifaction (fig. 3).

PRIMARY ELEMENTS.—The protoxylem can only appear in extremely well cut sections, but the metaxylem is conspicuous. About twenty metaxylem areas surround the small pith (fig. 3). In radial section the primary elements are seen to have spiral thickenings on their walls (fig. 11).

COMPARISON OF C. JURASSICA WITH LIVING CONIFERS

Cupressinoxylon jurassica was compared with species of the living genera *Taxodium, Sequoia, Libocedrus, Juniperus, Thuja, Chamaecyparis*, and *Cupressus*. A comparative microscopic eye-piece, permitting examination of two sections at the same time, was used in making most of the comparisons.

In *Juniperus* and *Libocedrus* the tangential walls of the ray cells are almost invariably thickened, while in *Cupressinoxylon jurassica* these walls are relatively thin. Further, in *Libocedrus* the pits on the lateral walls of the ray cells are simple or with an inconspicuous border. In *Taxodium* and *Sequoia* the bordered pits in the radial walls of the tracheids are multiseriate; in *C. jurassica* they are strictly uniseriate. Further, in *Taxodium* the cross walls of the parenchyma cells are greatly thickened and the pits in the radial walls of the tracheids are characteristically crowded, flattened, and often irregularly arranged(RECORD 21).

Cupressinoxylon jurassica was also compared with descriptions (sections for microscopic study not being at hand) of *Podocarpus, Thujopsis, Cryptomeria, Saxegothaea, Fitzroya, Actinostrobus, Widdringtonia, Callitris*, and *Arthrotaxis* as given by PRILL (19) and PENHALLOW (16). A review of the differences between these genera and *C. jurassica* will not be given, but it should be stated that in all cases some structural variation is indicated. So far as the living Cupressineae are concerned, *C. jurassica* most closely resembles *Cupressus* and *Chamaecyparis*.

In general the tangential ray cell walls of *Cupressinoxylon jurassica* are curved, agreeing with *Cupressus* (15). PENHALLOW (16) states that in *Chamaecyparis lawsoniana, C. pisifera*, and *C. obtusa* the tangential walls of the ray cells are thin and entire, while in *Cupressus macrocarpa, C. arizonica, C. macnabiana, C. goveniana, Chamaecyparis thyoides*, and *C. nootkatensis* the walls are somewhat thickened. The tangential ray cell walls in *C. jurassica* being relatively thin and entire, agree in this respect with the former group.

In both *Cupressus goveniana* and *C. macnabiana* the number of rays per square millimeter of tangential surface is much greater than in *Cupressinoxylon jurassica*. In the two former species the rays are much lower as a rule, and the pitting on the lateral walls

of the rays smaller in size than in *C. jurassica*, but of the same general type. In *Chamaecyparis nootkatensis* the number of rays per square millimeter is higher than in *C. jurassica*, but the difference is not so marked as in species of *Cupressus*. The ray cell pitting of *Chamaecyparis* is similar to that in *C. jurassica*, but the pits of *Chamaecyparis* are somewhat smaller.

The occasional occurrence of ray tracheids in certain species of both *Cupressus* and *Chamaecyparis* reported by JONES (7), PENHALLOW (15, 16), and JEFFREY (6) is not observed in *C. jurassica*. PENHALLOW (16) states that in *Chamaecyparis pisifera* and *Cupressus macrocarpa* pits in the radial walls of the tracheids are in one row or in pairs; the remaining species, *Chamaecyparis obtusa*, *C. lawsoniana*, *C. nootkatensis*, *C. thyoides*, *Cupressus macnabiana*, *C. arizonica*, and *C. goveniana* having strictly uniseriate pits. *C. jurassica* resembles the latter group in this respect.

JONES (7) figures a transverse section of *Cupressus macrocarpa*, showing peripheral groups of parenchymatous tissue which appear very similar to the development of wound tissue in *C. jurassica*. It appears, therefore, that *C. jurassica* bears considerable resemblance to the living genera *Cupressus* and *Chamaecyparis*. This appears in size and arrangement of the elements, as well as in more specific characters such as ray cell pitting.

One interesting difference between *C. jurassica* and living species of *Cupressus* and *Chamaecyparis* from Arizona, California, and southwestern Alaska is the greater density of the summer wood of the latter genera. Not only is it of greater density, but the boundary between the spring and summer wood of each growth ring is much sharper. A brief annual period of rest is indicated in the Como stems by the rather sharp transition from one annual growth ring to the next. A wet spring, dry late summer, warm wet late fall, and short dry, even frosty winter, would explain the *C. jurassica* rings. It seems reasonable that the late Jurassic climate was characterized by a less marked seasonal variation than prevails in the California coast region of North America at present.

Cupressus, with ten or twelve species, is confined to the Pacific Coast region of North America and Mexico in the New World, and to southwestern Europe, southwestern Asia, the Himalayas, and China in the Old World (PILGER 17, SARGENT 22). *Chamaecyparis*,

with six species, is confined to the Atlantic and Pacific Coast regions of North America, and to Japan and Formosa (17, 22).

COMPARISON OF C. JURASSICA WITH FOSSIL CONIFEROUS WOODS

Cupressinoxylon is briefly described by KRÄUSEL (11). A large number of fossil coniferous stems have been referred to this genus. KNOWLTON (8) lists twenty-seven species of *Cupress(in)oxylon*, together with one species each of *Cupressus* and *Cupressites* from North America. KRÄUSEL (9) records over 170 specimens, which were variously referred to *Cupressinoxylon*, *Cupressoxylon*, *Cupressus*, and *Cupressites*. In many cases the generic names *Cupressinoxylon* and *Cupressoxylon* have been used synonymously in describing the same species, many of the specimens were not determined as to species, and several of them were question marked. Some of the determinations were based on poorly preserved or scanty material, so that it is not surprising that the identifications cannot be authenticated. KRÄUSEL (9) limits the number of forms which may be referred with reasonable certainty to *Cupressinoxylon* to seventeen species, as follows:

Species	Age	Occurrence
1. Cupressinoxylon wellingtonioides	Miocene	Silesia
2. C. cupressoides	Miocene	Silesia
3. C. gothani	Miocene	Silesia
4. C. patagonicum	Oligocene	Patagonia
5. C. sylvestre	Tertiary	Russia
6. C. severzowi	Tertiary, Cretaceous (Greensand)	Russia
7. C. sanguineum	?	Russia
8. C. sp. Kräusel	Miocene	Silesia
9. C. sp. Gothan	Tertiary	Spitzbergen
10. C. sp. Prill	Miocene	Silesia
11. Cupressoxylon podocarpoides	Tertiary (also Cretaceous?)	Japan
12. Cupressus pritchardi	Eocene	Ireland
13. Rhizocupressinoxylon liasinum	Liassic	Orne, France
14. Libocedrus sabiniana	Tertiary	Greenland
15. Sequoia canadensis	Miocene	Canada
16. S. magnifica	Tertiary	Yellowstone Park, North America
17. Biota orientalis var. miocenica	Miocene	Silesia

STOPES (24) lists four species of *Cupressinoxylon* as follows:

1. Cupressinoxylon vectense	Lower Greensand	Isle of Wight
2. C. luccombense	Lower Greensand	Isle of Wight
3. C. cryptomerioides	Kentish Rag	Maidstone
4. C. hortii	Lower Greensand	Woburn

Cupressinoxylon jurassica was compared with these species and found to differ in every case. The chief differences noted are as follows.

1. *Cupressinoxylon wellingtonioides*, according to the description given by PRILL and KRÄUSEL (20), differs from *C. jurassica* chiefly in the greater height of its rays (a maximum of thirty-five cells being noted) and the commonly biseriate character of the pits on the radial walls of the tracheids. Further, the rays of *C. wellingtonioides* appear to be largely uniseriate and small vertical resin canals appear in the wound tissue.

2. *Cupressinoxylon cupressoides* differs from *C. jurassica* chiefly in that its parenchyma cells occur in two to three tangential bands. The pitting on the radial walls of the tracheids is biseriate in part, and pits on the tangential walls of the tracheids are numerous. There may be as many as two pits per tracheid field on the lateral walls of the ray cells. The rays are usually low, the highest having twenty-three cells; seldom are they biseriate (9, 10).

3. *Cupressinoxylon gothani* (9, 10) seems to be similar to *C. jurassica* in some respects. The chief differences lie in the somewhat higher maximum heights of the rays of *C. jurassica* and in the greater tendency for the tangential walls of the ray cells to be curved in *C. jurassica*. KRÄUSEL (10) shows a commonly biseriate condition of pitting. In this respect also it differs from *C. jurassica*.

4. *Cupressinoxylon patagonicum* is stated by KRÄUSEL (9) to be a coniferous wood without resin canals, belonging to *Cupressinoxylon*, but which cannot be determined more closely.

5. *Cupressinoxylon sylvestre* differs from *C. jurassica* in several particulars. FELIX (2) quotes MERCKLIN (14) as describing a concentric grouping of the parenchyma cells in *C. sylvestre*. MERCKLIN stated that the rays varied from two to twelve cells in height.

6, 7. *Cupressinoxylon severzowi* cannot be distinguished from *C. sanguineum* according to KRÄUSEL (9). The latter species is a coniferous wood without resin canals, that cannot be determined

more closely. Possibly it may belong to *Cupressinoxylon*. *C. jurassica* cannot be carefully compared with either of these species.

8–10. *Cupressinoxylon* sp. Kräusel was not determinable as to species (**9, 10**), due to poor preservation of the material. The maximum height of the rays is given as twelve cells. *C.* sp. Gothan and *C.* sp. Prill both represent specimens which in consequence of their poor preservation cannot be determined as to species (**9**).

11. *Cupressoxylon podocarpoides* is stated (**9**) to be a coniferous wood without resin canals, which possibly belongs to *Cupressinoxylon*, but which cannot be determined more closely because of insufficient description.

12. *Cupressus pritchardi* is, in the words of KRÄUSEL (**9**), too briefly described for certain determination. GARDNER (**3**) bases his description on vegetative characters and cones, and MACLOSKIE (**13**) figures only a small radial view of the wood. In MACLOSKIE'S figure the tangential walls of the ray cells are not shown, and the pitting on the lateral walls appears to consist of large simple pits, or if bordered, the pore has been omitted.

13. *Rhizocupressinoxylon liasinum* is considered to be the oldest true *Cupressinoxylon* by GOTHAN (**4**). KRÄUSEL (**9**) states that the arrangement of the pitting of the tracheids in this species is not entirely clear. LIGNIER (**12**) states that there are thirty-six rays per square millimeter of tangential surface. The average number per square millimeter in *Cupressinoxylon jurassica* is about fifteen. The rays of *R. liasinum* are considerably lower than in *C. jurassica*, and the number of pits per tracheid field on the lateral walls of the ray cells is from three to five, which is somewhat higher than in *C. jurassica*. In addition, the pitting on the lateral walls of the tracheids is biseriate in *R. liasinum*.

14. *Libocedrus sabiniana* cannot be determined as to species with certainty, since the structure of the medullary rays is not clear (**9**).

15. *Sequoia canadensis* cannot be carefully compared with *C. jurassica* since it is impossible to make a closer determination than to say that the specimen belongs to *Cupressinoxylon* (**9**).

16. *S. magnifica*, according to PENHALLOW (**16**), has biseriate pitting, at least in part, on the radial walls of the tracheids. Due to poor preservation of the specimen, KRÄUSEL (**9**) states that it

cannot be determined more closely than as belonging to *Cupressinoxylon*.

17. *Biota orientalis* var. *miocenica*, according to KRÄUSEL (9), is a typical cupressinen wood whose peculiar "intercellulars" point with certainty to *Biota*. In *C. jurassica* the "intercellulars," or what only appear to be such, are normal.

Of the four species of *Cupressinoxylon* listed by STOPES (24) from the English Lower Greensand, all differ specifically from *C. jurassica*, although *C. vectense* is nearest, and has the complex growth rings, but with much lower wood rays. *C. cryptomerioides* has slight growth rings and quite low wood rays; *C. luccombense* has low wood rays and variant ray pitting; *C. hortii* has wood rays of extraordinary abundance, height, and width, being perhaps separated generically from other *Cupressinoxylon*.

RELATIVE AGE OF SPECIES HAVING CUPRESSINEAN AFFINITIES

In view of the age of *Cupressinoxylon jurassica*, it is of interest to note the ideas held as to the age of species having cupressinean affinities. SEWARD (23) states: "It is undoubtedly true that in the later Jurassic and Lower Cretaceous floras, conifers agreeing generally in habit and in the possession of appressed imbricate leaves with such genera as *Cupressus*, *Chamaecyparis*, and *Thuya* were among the most characteristic types." JEFFREY (5) states that "all evidence goes to show that the Taxodineae and Cupressineae did not exist before the very end of the Cretaceous or more probably before the beginning of the Tertiary." COULTER and CHAMBERLAIN (1) state that although *Cupressus*-like twigs and cones have been described from the Jurassic, there is no reliable evidence of the tribe earlier than the Upper Cretaceous. POTONIÉ and GOTHAN (18) state that *Cupressinoxyla*, *Glyptostroboxyla*, and *Taxodioxyla* are common in the Tertiary as "Braunkohlenhölzer."

Summary

A new species, *Cupressinoxylon jurassica*, from the late Jurassic of South Dakota, is described and figured. This species is the oldest that has been certainly referred to *Cupressinoxylon*. The determination is based on microscopic study of ten thin sections and compari-

son with fossil and living coniferous woods. A strictly modern conifer type, in all respects, is indicated.

The writer wishes to acknowledge the aid of Dr. G. R. WIELAND in this study, particularly in the interpretation of the seasonal annulation of the Como stems.

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CYTOLOGICAL STUDIES ON THE BETULACEAE

IV. BETULA, CARPINUS, OSTRYA, OSTRYOPSIS

ROBERT H. WOODWORTH

(WITH THIRTY-FOUR FIGURES)

It has been pointed out in the previous papers of this series (WOODWORTH 4, 5) that polymorphism in the Betulaceae is due, in part at least, to the readiness with which the species cross in nature. It has also been noted that certain irregularities of the reduction division leading to polycary, polyspory, sterile pollen, parthenogenesis, apogamy, polyembryony, polyploidy, and polymorphism suggest a heterozygous origin for plants showing any of these characters (WOODWORTH 6).

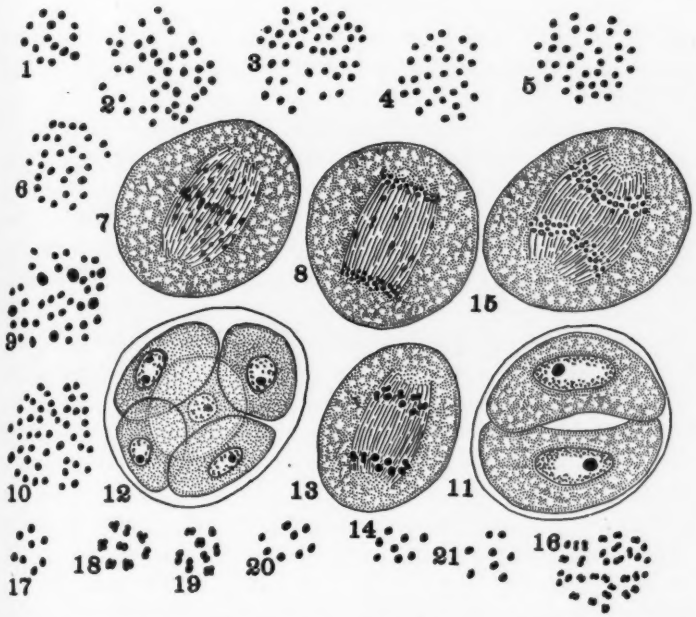
Materials and methods are explained in the first paper of this group (4). Professor C. O. ROSENDAHL has very kindly sent me collections from the birches growing in Minnesota.

BETULA (CONTINUED)

Betula utilis var. *prattii* Burk, X-14 (meiosis normal).—Fig. 1 shows a metaphase plate of the first division with fourteen chromosomes.

B. lutea Michx. f., X-42 (meiosis normal).—The material reported on previously by the writer (4) was collected in Massachusetts. The material here dealt with is from Minnesota. The meiosis is identical and shows the species in both regions to be hexaploid. Fig. 2 shows a metaphase plate of the first division with forty-two chromosomes. It was interesting to find that this is so, because *B. lutea* is one of the parents in × *B. purpusii* treated in this paper.

B. papyrifera Marsh. and its varieties are most interesting because of their extreme polymorphism. True *B. papyrifera* and the variety *cordifolia* have been found to have thirty-five (pentaploid) and twenty-eight (tetraploid) respectively as the reduced number of chromosomes (4). Three other varieties are reported here. Such work on the morphology of the chromosome groups may help in untangling their polymorphism.



FIGS. 1–21.—Fig. 1, *Betula utilis* var. *prattii* Burk., metaphase plate of first division showing 14 chromosomes. Fig. 2, *B. lutea* Michx. from Minnesota, metaphase plate of first division showing 42 chromosomes. Fig. 3, *B. papyrifera* var. *occidentalis* Sarg., heterotypic metaphase plate showing 42 chromosomes. Fig. 4, *B. papyrifera* var. *subcordata* Sarg., heterotypic metaphase plate showing 28 chromosomes. Fig. 5, *B. papyrifera* var. *kenaica* Henry, heterotypic metaphase plate showing 35 chromosomes. Fig. 6, *B. pumila* var. *glandulifera* Regel, heterotypic metaphase plate showing 28 chromosomes. Figs. 7–12, × *B. purpusii* Schneid. (*B. pumila* var. *glandulifera* × *B. lutea*): fig. 7, metaphase of first division showing many univalent chromosomes; fig. 8, anaphase of first division showing lagging univalent chromosomes; fig. 9, heterotypic metaphase plate showing 36 vari-sized chromosomes; fig. 10, heterotypic metaphase plate showing 47 chromosomes; fig. 11, diad of diploid pollen grains; fig. 12, polyspory, five pollen grains rather than normal tetrad. Figs. 13, 14, *Carpinus betulus* L.: fig. 13, anaphase of first division showing 8 chromosomes; fig. 14, heterotypic metaphase plate showing 8 chromosomes. Figs. 15, 16, *C. betulus* var. *fastigiata* Nichols: fig. 15, anaphase of first division showing 32 chromosomes; fig. 16, heterotypic metaphase plate showing 32 chromosomes. Fig. 17, *C. caroliniana* Walt., heterotypic metaphase plate showing 8 chromosomes. Fig. 18, *C. laxiflora* Bl., diakinesis showing 8 pairs of chromosomes, several split for second division. Fig. 19, *C. turczaninovii* Hance., diakinesis showing 8 pairs of chromosomes, one pair already split for second division. Fig. 20, *C. orientalis* Mill., heterotypic metaphase plate showing 8 chromosomes. Fig. 21, *C. japonica* Bl., heterotypic metaphase plate showing 8 chromosomes.

B. papyrifera var. *occidentalis* Sarg., X-42 (meiosis normal).—This variety differs from the true species and the variety just noted in being hexaploid. Fig. 3 shows the metaphase plate of the first division with forty-two chromosomes.

B. papyrifera var. *subcordata* Sarg., X-28 (meiosis normal).—Fig. 4 shows twenty-eight chromosomes in the heterotypic metaphase plate. This variety is tetraploid.

B. papyrifera var. *kenaica* Henry, X-35 (meiosis normal).—This is the only variety of the four studied which is pentaploid like *B. papyrifera*. Fig. 5 is the heterotypic metaphase plate with thirty-five chromosomes.

B. pumila var. *glandulifera* Regel, X-28 (meiosis normal).—Fig. 6 shows the metaphase plate of the first division with twenty-eight chromosomes. When noting the decided correlation of results of ROSENDAHL'S (3) taxonomic work and the writer's cytological work on × *B. sandbergi* (*B. papyrifera* × *pumila* var. *glandulifera*) (4), the *B. pumila* var. *glandulifera* parent had not been available for investigation. *B. pumila* was found to have twenty-eight pairs of chromosomes, and the chromosome counts of *B. sandbergi* indicated that the variety must have the same number as the true species. This is now seen to be correct. The material used was sent from Minnesota by Professor ROSENDAHL. This variety is one of the parents of the hybrid birch discussed just below.

× *B. purpusii* Schneid. (*B. lutea* × *pumila* var. *glandulifera*), 2X-70; X about 45 (meiosis very abnormal).—The cytological study of this hybrid has proved to be a distinct correlation and conclusion to the taxonomic studies of ROSENDAHL (3), whose observations extended over several years. His drawings of the leaves, catkins, cone scales, and seeds show *B. purpusii* to possess characters which are intermediate, in every way, between those of the swamp birch and the yellow birch. He points out that there are but three species of *Betula* in Minnesota, where he found this hybrid. These are *B. papyrifera*, *B. pumila* var. *glandulifera*, and *B. lutea*. Hybrids between the first two of these have already been described taxonomically by ROSENDAHL (3) and cytologically by the writer (4). In the taxonomic work, photomicrographs of pollen of the three species show it in each case to be 100 per cent perfect. The two hybrids show 33 and 45 per cent of the pollen defective.

The *lutea* parent of this hybrid has forty-two as the reduced number of chromosomes. The *pumila* var. *glandulifera* parent has twenty-eight. Metaphase plates of sporophytic cells in the hybrid contain about seventy chromosomes. The latter are so small and so close together that an accurate count is almost impossible.

The nuclei in diakinesis contain so many chromatic elements that they are most difficult to study. It appears that a majority of the chromosomes from the *glandulifera* parent find synaptic mates and appear during the first division as bivalent chromosomes. Those which do not pair and the extra fourteen or more from the *lutea* parent lag on the spindle. Fig. 7 shows the metaphase of the first division. Pollen mother cells in the phase viewed from one of the poles show from thirty-six (fig. 9) to forty-seven (fig. 10) chromosomes. In cases where there are less than forty-two, some of the *lutea* chromosomes may have paired among themselves, or several chromosomes may have fused into one mass, as fig. 9 suggests (WOODWORTH 5). In the anaphase of the first division the chromosomes which did not pair are very tardy in their movements on the spindle (fig. 8). The second division is also typified by such random movements of the chromosomes. Frequently a number of these laggards are not included in the nuclei at the spindle poles and they form extra pollen grains (fig. 12).

At times the tardiness is so marked that all the chromosomes on the spindle are included in one nucleus, which then forms a broad spindle and produces a diad of diploid pollen grains (fig. 11). This is the semiheterotypic division (ROSENBERG 2) which was also found in *B. sandbergi* and other species (WOODWORTH 4). This production of polyploid gametes is now becoming better understood (KARPECHENKO 1). It is an excellent explanation of hybridity as an important cause of polyploidy.

Many of the anthers show cytomyxis and chromatolysis to a marked degree. This was found to be the case in other species which were probably of hybrid origin (WOODWORTH 4). Occasionally a long string of pollen mother cells is seen to be fused together. From one-third to one-half of the pollen is morphologically sterile.

All species of *Betula*, *Alnus*, and *Corylus* studied by the writer (4, 5) have fourteen as the fundamental number of chromosomes.

The other three genera, *Carpinus*, *Ostrya*, and *Ostryopsis*, have eight as the fundamental number.

CARPINUS (TOURN.) L.

Carpinus betulus L. X-8 (meiosis normal).—Figs. 13 and 14 represent the anaphase and metaphase plate of the first division. Both show eight chromosomes.

C. betulus var. *fastigiata* Nichols, X-32 (meiosis normal).—This variety is an octoploid form. The pollen mother cells are accordingly several times larger than those of *C. betulus*. Figs. 15 and 16 show the anaphase and metaphase plate of the first division with thirty-two chromosomes.

C. caroliniana Walt., X-8 (meiosis normal).—Fig. 17 shows eight chromosomes in the heterotypic metaphase plate.

C. laxiflora Bl., X-8 (meiosis normal).—Fig. 18 represents the diakinesis with eight pairs of chromosomes, some of which are split for the second division.

C. turczaninovii Hance, X-8 (meiosis normal).—Fig. 19 shows the diakinesis with eight pairs of chromosomes, one pair already split for the second division.

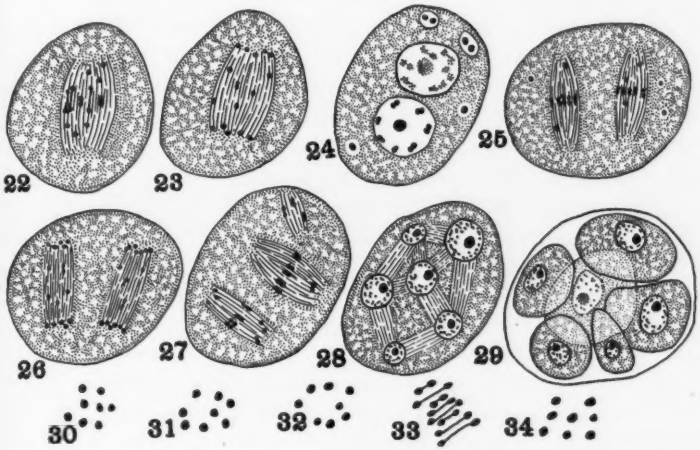
C. orientalis Mill., X-8 (meiosis normal).—Fig. 20 shows eight chromosomes in the heterotypic metaphase plate.

C. japonica Bl., X-8 (meiosis normal).—Fig. 21 is the heterotypic metaphase with eight chromosomes.

C. cordata Bl., X-8 (meiosis very abnormal).—The diakinesis is an easy stage to study because there are so few chromosomes. Characteristically several of the chromosomes do not pair. These appear on the spindle as univalents, as in the early anaphase shown in fig. 22. In the late anaphase they lag on the spindle (fig. 23). Some of the laggards do not enter the interkinetic nuclei but form micronuclei (fig. 24). The homeotypic division is also very irregular. Fig. 25 shows the metaphase with lagging and extruded chromosomes. The anaphase shows these laggards (fig. 26). Fig. 27 portrays an anomalous homeotypic division with three abnormal spindles, the extra spindle apparently having been formed by extruded chromosomes. These abnormalities lead to the formation of polycaric mother cells. Fig. 28 shows such a cell with six nuclei rather than the normal tet-

rad. Polyspory results (fig. 29). Large numbers of the pollen grains degenerate.

In studying the cytology of the several species comprising a genus, it is interesting to find most of the species passing through the meio-



FIGS. 22–34.—Figs. 22–29, *Carpinus cordata* Bl.: fig. 22, early anaphase of first division showing unpaired chromosomes; fig. 23, late anaphase of first division showing lagging univalent chromosomes; fig. 24, interkinesis: nuclei have 6 and 7 chromosomes respectively, the other 3, already split, reposing in cytoplasm; fig. 25, homeotypic metaphase showing lagging and extruded chromosomes; fig. 26, homeotypic anaphase showing lagging chromosomes; fig. 27, anomalous homeotypic division with three abnormal spindles; fig. 28, polycaric mother cell showing 6 nuclei; fig. 29, polyspory, 6 pollen grains instead of normal tetrad. Fig. 30, *Ostrya virginiana* K. Koch., heterotypic metaphase plate showing 8 chromosomes. Fig. 31, *O. virginiana* var. *glandulosa* Sarg., heterotypic metaphase plate showing 8 chromosomes. Fig. 32, *O. japonica* Sarg., heterotypic metaphase plate showing 8 chromosomes. Fig. 33, *O. carpinifolia* Scop., early anaphase of first division showing 8 chromosomes. Fig. 34, *Ostryopsis davidiana* Dcne., heterotypic metaphase plate showing 8 chromosomes.

tic division in a wholly normal manner, and one of the species possessing the meiotic irregularities just discussed. Since the materials from all of the plants were collected at the same time, and treated throughout the technical procedure in exactly the same manner, it is obvious that there is an innate unbalance in the cells themselves. The results of these abnormalities, polycary and polyspory, show

that the phenomenon is a natural one having nothing to do with the fixative. In fact, these irregularities can be seen in living material.

Carpinus cordata (that is, the plants growing in the Arnold Arboretum) shows the meiosis which is well known to be typical of hybrid plants, and is therefore put under suspicion of having arisen by heterozygosis. There is a very interesting particular concerning the bract which subtends the nutlets. In all other species of *Carpinus* this bract is flat. In *Ostrya* species the nutlet is inclosed in a tubular involucre open at the top. The bract of *Carpinus cordata* is not flat as in the other species of the genus, but is inflexed at the base covering the nutlet. This advances the possibility that *C. cordata* may be a bigeneric hybrid between *Carpinus* and *Ostrya*.

Ostrya (Mich.) Scop.

Ostrya virginiana K. Koch., X-8 (meiosis normal).—Fig. 30 shows the heterotypic metaphase plate with eight chromosomes.

O. virginiana var. *glandulosa* Sarg., X-8 (meiosis normal).—Fig. 31 shows the heterotypic metaphase plate with eight chromosomes.

O. japonica Sarg., X-8 (meiosis normal).—Fig. 32 shows the heterotypic metaphase plate with eight chromosomes.

O. carpinifolia Scop., X-8 (meiosis normal).—Fig. 33 shows the early anaphase of the first division with the eight pairs of chromosomes just separating.

Ostryopsis Dcne.

Ostryopsis davidiana Dcne., X-8 (meiosis normal).—Fig. 34 shows the heterotypic metaphase plate with eight chromosomes.

Summary

1. *Betula lutea* from Minnesota is hexaploid as is the New England plant.

2. *B. papyrifera* and its varieties have interesting chromosome numbers. *B. papyrifera* has the haploid number of thirty-five; *B. papyrifera* var. *cordifolia* has twenty-eight; *B. papyrifera* var. *subcordata* has twenty-eight; *B. papyrifera* var. *kenaica* has thirty-five; *B. papyrifera* var. *occidentalis* has forty-two. It is hoped that this will help to explain the polymorphism in the group.

3. *B. pumila* var. *glandulifera* has twenty-eight pairs of chromosomes.

4. × *B. purpusii* (*B. lutea* × *pumila* var. *glandulifera*) presents another complete document, taxonomically and cytologically, of natural hybridization.

5. *Carpinus*, *Ostrya*, and *Ostryopsis* species have eight as the fundamental number of chromosomes.

6. *Carpinus betulus* var. *fastigiata* is octoploid with thirty-two chromosomes as the reduced number.

7. *Carpinus cordata* shows all the cytological characteristics of a hybrid and is accordingly suspected of having a heterozygotic ancestry. It may be a bigeneric hybrid.

8. The existence of polyploidy and irregular meioses in polymorphic plants, as here reported, furnishes more evidence for the theory that multiplication of species has come about to a considerable extent by hybridization.

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BRIEFER ARTICLES

IS FASCIATED A FREQUENTLY MUTATING CHARACTER?

The fasciation of *Pharbitis nil*, the Japanese morning glory, is manifested by certain polymeric genes. The writer[1] has already described three genes, fasciated-1, fasciated-2, and fasciated-3. Owing to the reduplicated nature of the genes on the one hand, and the possible existence of modifiers on the other, the segregating proportions of fasciated to normal are often complicated and deviate from the expectations. Some genotypically fasciated species have uncertain manifestations of fasciation; in other words, the fasciated pedigrees frequently give some normal atavists among their progeny, sometimes containing even a majority of normals. HAGIWARA[2] regarded them as produced by gene mutation. On selfing 5 fasciated plants obtained in hybrid progeny, he got 119 individuals containing 41 atavists.[3] He also called attention to the occurrence of reverse mutations from normal to fasciated. This possibility, however, is based on rather unconvincing facts. Of course, our fasciated species originated spontaneously from normal ancestors, and this origin would surely be due to mutation. This mutation, however, seems to be very rare. As to the occurrence of normal atavists among fasciated sisters, YAMAGUCHI[4] also reported as follows:

Was zunächst das oftmalige Auftreten der Atavisten (Rückmutanten) in den verschiedenen Linien mit fasziiertem Stengel anbetrifft, so scheinen mir darunter zwei Kategorien unterschieden werden zu sollen. Die Atavisten, die nach der Selbstbefruchtung ausschliesslich fasziierte Nachkommen geben, wie ich bisher beweisen konnte, sollten der einen Kategorie angehören, während sie

[1] IMAI, Y., Experiments with a pear-leafed and fasciated strain of the Japanese morning glory. Jour. Genetics **18**:275–314. 1927.

[2] HAGIWARA, T., Genetic studies of the fasciation in morning glories. Bot. Mag. Tokyo **40**:281–294. 1926 (Japanese).

[3] Most of them were noted as having non-pear leaves among otherwise pear leaves. To the writer, such non-pear and non-fasciated plants would have suggested contamination rather than double mutation.

[4] YAMAGUCHI, Y., Notiz über die Vererbung der Fasziation bei *Pharbitis nil*. Bot. Mag. Tokyo **40**:535–537. 1926.

sich bei der anderen wieder in die ihr ähnlichen normalen und fasziierten zu spalten pflegen. Die erste Kategorie könnte man etwa falsche Atavisten (Pseudo-Rückmutanten bzw.-Rückmutation) nennen, während es sich bei der zweiten um die echten Atavisten, d.h. um die Rückmutation des Gens handelt.

In other words, YAMAGUCHI accepted HAGIWARA'S view that fasciated is a frequently mutating character, but at the same time he recognized its fluctuation resulting in a normal character. YAMAGUCHI'S paper being in the nature of a short note, he did not give numerical data supporting his view.

The writer, however, concluded that these normal atavists are due merely to fluctuation in manifestation of the character fasciated. His evidence was furnished by the hybrid progeny derived from a cross between non-fasciated no. 326 and fasciated no. A5. An F_3 examination of this cross covers progenies of 183 F_2 plants, of which 35 are pear and non-fasciated and 5 are pear and fasciated. Of these 35 pear and non-fasciated F_2 plants, two proved, on examination of their offspring, to be really pear and fasciated; that is, they were false normals. Combining these results with those of properly fasciated plants, the data contain 65 individuals, of which 6 are normal atavists. When the previous paper was written, these were all the data available to support the writer's opinion that atavists are due merely to the fluctuating manifestation of the character fasciated. In the successive years two further generations of fasciated F_3 plants have been raised to prove this view and settle the problem.

On selfing two fasciated F_3 plants of pedigree no. 169, of which the F_2 mother plant was a false normal (atavist), the writer obtained F_4 offspring consisting of 16 fasciated and 10 normal plants and 33 fasciated and 9 normal plants respectively. From the former pedigree an F_5 generation was reared. The F_5 offspring of 16 fasciated F_4 consisted of 162 fasciated and 42 normal plants, and those of 9 normal F_4 contained 66 fasciated and 17 normal plants. The proportions of normal atavists in these pedigrees are 21 per cent in the former and 20 per cent in the latter, giving practically the same results. These data collectively do not furnish anything for the mutation view, but give indisputable evidence for the fluctuation view. The genes for fasciated, therefore, are of constant state, and the frequent appearance of normal atavists among fasciated sisters is due to a fluctuating representation. These atavists are of a temporary form. Returning to HAGIWARA'S data, they do not give any positive evidence for his mutation view. For YAMAGUCHI'S view we cannot argue beyond his statement, because he did not present numerical data.

The proportion of false normals among fasciated sisters is very variable, owing partly to various genotypes of the fasciated complex and partly to the fluctuating manifestation of the fasciated character. One of the writer's fasciated strains, no. A5, is very fixed for the representation of the characteristic and contains almost no atavists. YAMAGUCHI[5] stated that his fasciated strains were also highly fixed for this character. In his extensive cultivation[6] of these pedigrees he obtained only 1 per cent of normal atavists among some 1200 or 1300. Another of the writer's fasciated strains gives about 15 per cent of false normals, although the proportion varies to some extent in the different cultures. The fasciated pedigrees obtained in the hybrid progeny are generally more unstable, through the interference of certain modifiers. The commercial strains, however, are generally of high fixation for the fasciated characteristic, because some intense selection would have been made before their registration.—Y. IMAI, *Botanical Institute, Agricultural College, Tokyo Imperial University.*

[5] YAMAGUCHI, Y., On the inheritance of fasciation in *Pharbitis nil.* Proc. Jap. Assn. Adv. Sci. **2**:264–273. 1926 (Japanese).

[6] Owing to the defective sexual organs fasciated strains generally give few seeds.

CURRENT LITERATURE

BOOK REVIEWS

Oedogoniaceae

The long awaited monograph of the Oedogoniaceae[1] by TIFFANY has appeared, and it justifies the hope that a great university, with three members of its botanical staff specializing in algae, would bring out a work of the first order. Foreign universities have long recognized the desirability of comprehensive monographs, written by specialists in the various groups; but in America such monographs have been more or less limited to the seed plants. Exceptions, like the monograph on *Isoetes* by PFIEFFER, are not numerous. Monographs like COLLIN'S *Green algae of North America*, while useful and stimulating, are too local.

For the past 30 years the principal monograph of the Oedogoniaceae has been HIRN'S *Monographie und Iconographie der Oedogoniaceen*. This work has become hard to secure, and, during the 30 years since its publication, many new species have been described and many have been emended. TIFFANY'S study of American and foreign forms, extending over a period of 15 years, has fitted him for undertaking an up-to-date monograph.

After a presentation of the well known facts of cell structure and life history, there is an interesting account of periodicity and distribution. In the north central United States the maximum of sexual reproduction in *Oedogonium* is reached in May and in July, in both annual and perennial species, with a second maximum, not nearly so vigorous, in October. In the same area 54 per cent of the species are found in permanent ponds, 23 per cent in lakes, 15 per cent in temporary ponds, 6 per cent in streams, and 2 per cent in stream oxbows. *Bulbochaete* shows a similar behavior. In *Bulbochaete* 42 species, two varieties, and one form are dioecious, nanandrous; six species, two varieties, and one form are monoecious; there are no dioecious macrandrous species. In *Oedocladium* three of the four species are monoecious and one dioecious. All are nanandrous. In *Oedogonium* 61 species, 15 varieties, and 8 forms are monoecious; 56 species, 25 varieties, and 11 forms are dioecious, macrandrous; and 77 species, 36 varieties, and 19 forms are dioecious, nanandrous.

It is hard enough to define a species, but varieties and forms are still more indefinite. As TIFFANY uses the terms, an alga which occurs year after year in

[1] TIFFANY, L. H., The Oedogoniaceae, a monograph including all the known species of the genera *Bulbochaete*, *Oedocladium*, and *Oedogonium*. 8vo. pp. 253. *pls. 64*. Published by the author. Columbus, Ohio. 1930.

the same habitat with a known species, but differs in one or more morphological characters, but is evidently closely related to the type, is a *variety*. If an alga differs from the description of a species, but has been seen only once or twice, one cannot know whether a variation is constant or is merely an ecological variation. The term *form* is used for such cases.

The keys are in English and are particularly definite; probably the average botanist who has had a course in the morphology of algae could use them. This statement cannot be made in regard to many keys in thallophytes where a genus has such a large number of species as is found in *Bulbochaete* and *Oedogonium*.

A monograph on the Zygnemaceae is in preparation, and it is hoped that others will follow.—C. J. CHAMBERLAIN.

Statistical methods for research workers

A third edition of FISHER's monograph[2] follows closely the publication of the second edition.[3] There is little difference between the second edition and the third. The chief change is made near the end of the final chapter on the principles of statistical estimation, where section 57 is followed by two new subsections on fragmentary data, and on the amount of information, design, and precision. These sections occupy 10 pages, and illustrate some wider applications of the method of maximum likelihood, and the quantitative evaluation of information. In the citations of sources used for data and methods at the end of the book, the author has removed his own contributions to a separate list, in which he includes practically all of his papers, whether pertinent or not.

It is doubtful whether a third edition was necessary at this time. There should be some compelling reason for multiplying editions of books, however good they may be.—C. A. SHULL.

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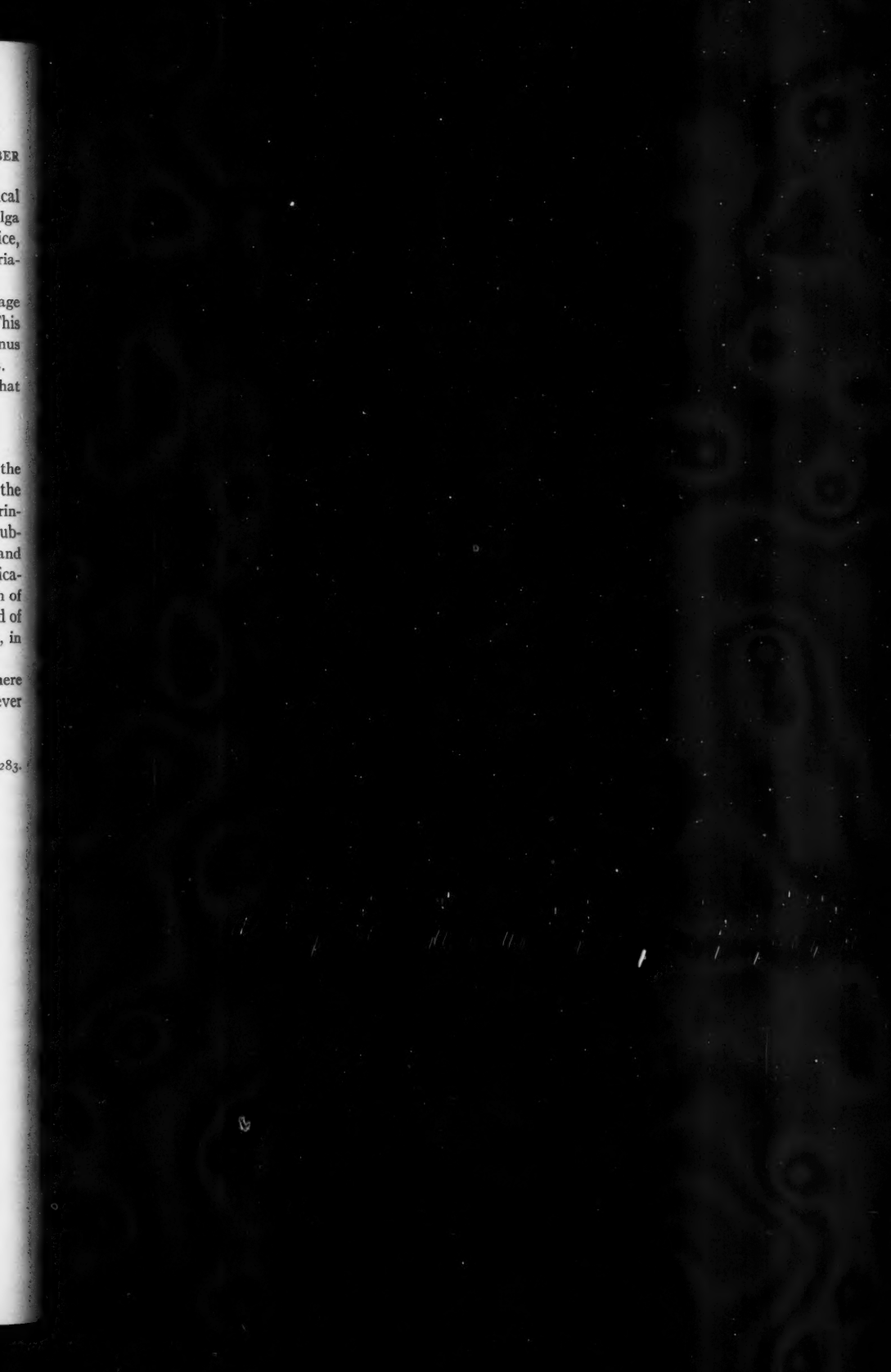
[2] FISHER, R. A., Statistical methods for research workers. 8vo. pp. xvi+283. Edinburgh and London: Oliver and Boyd. 1930.

[3] BOT. GAZ. **87**:670–671. 1929.